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INVESTIGATIONS
INTO
D'HERELLES PHENOMENON

BY

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C. Holten.
(L. S.)

Goos.

INVESTIGATIONS

INTO

D'HERELLES PHENOMENON

Das Studium von Bacteriophagen ist für den, der es beginnt, einige einzige Kette von geradezu aufregenden Überraschungen aber auch der, welcher durch lange eigne Untersuchungen einigermassen abgehärtet ist, sieht sich immer vor neue Rätsel gestellt.

BAIL.

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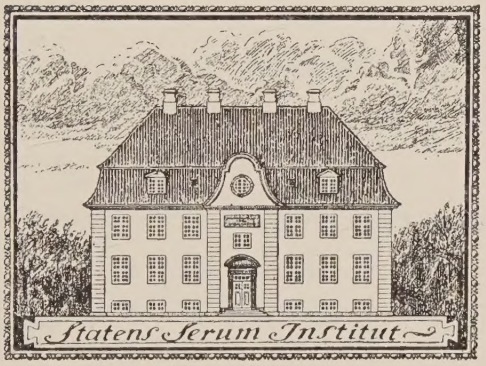

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Denne Afhandling er af det medicinske Fakultet antagen til offentlig at forsvares for den medicinske Doktorgrad.

Den 4. Juli 1925.

V. BIE
D. A. DEKANUS

H. H. THIELES BOGTRYKKERI. KØBENHAVN

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IT was not until September 1920 that I came face to face with d'Herelle's phenomenon for the first time, Dr. Madsen having procured from Bordet a lysin with the corresponding coli strain. At Dr. Madsens request I took up the matter for investigation.

The phenomenon itself, that the filtrate of fecal suspension was capable of dissolving bacteria and that this dissolution might be continued indefinitely by cultivation in series was in itself of considerable interest. It would, however, become considerably further-reaching if the assumption could be upheld that the lysin consisted of an ultravisible virus living on the bacteria. If from this virus conclusions could be drawn as to other ultravisible vira, there would be a possibility of progressing in the study of these and the diseases they produce.

The main question, however, bacteriolysis and the thereto appertaining problems, proved gradually as investigations proceeded to become so comprehensive that they alone took up all the time at our disposal.

From d'Herelles initial publication in 1917 and until 1920 reports were very sparse. But from the end of 1920 to date the problem has occupied bacteriologists all over the world and has been one of the most frequently recurring topics in all bacteriological and many medical periodicals.

The queries brought into being by the study of d'Herelles phenomenon gradually became legion, some were more specialized, others simply bacteriological. To render an exhaustive account of the results of the research work done, to say nothing of the experimental revision of the main problems alone, gradually proved to be outside the scope of this work.

The steady progress of the work was constantly disturbed by reports as to entirely new points concerning the matter in question, reports that frequently confronted one with problems that it was felt

ought to be taken up in order to penetrate more deeply into the subject one at the time was studying. Frequently by this means a deviation from the mainline of ones own investigations resulted and even though experience of great value was gained, much of the work done did not lead to results corresponding to the efforts.

The investigations reported in the experimental part of this work may possibly appear rather scattered, but this is due to the great manysidedness of the problem.

Much of the work done has not been communicated, partly because it lead to no result, taking the questions posed into consideration, even though they gave a certain amount of information of a more peripherial significance, partly because prior to their accomplishment they proved to be without aim owing to the results of the other investigations.

I must here express my sincerest gratitude to Dr. Madsen, who at the State Serum Institute of Copenhagen gave me every assistance and the incentive to take up this work.

THE MAIN PROBLEMS ELUCIDATED BY THE LITERATURE.

A historical summary in chronological order, where the whole period covers only about 7 years, would scarcely be of great interest.

Many will perhaps, on the assumption that the question of priority is of special importance, regard this point of view as erroneous. I do not share this view, for the consciousness and gratification of having accomplished a task is independent of whether others have come to the same results by similar or different ways and means.

Regarding the problems dealt with here among which occur several phenomena never before observed, the confirmation or invalidation — may be seen from entirely new viewpoints — frequently plays quite as important a rôle as the work upon which the investigation is based.

Historically viewed the judging as to whether the phenomenon observed by d'Herelle is identical with certain previously made observations might be of some interest.

The earliest observation I have had occasion to note is that of Hankins in 1896.

He noted that water from certain rivers in India exercised a pronouncedly antiseptic effect on bacteria in general and on the cholera vibrio in particular. The water from the river Jumna thus contains on leaving the town of Agra more than 100,000 bacteria per cc. whereas 5 kilometers lower down it contains 90 to 100. In vitro too it has a pronounced germicidal action. This action disappears when the water is boiled, consequently Hankin is of the opinion that the germicidal matter is volatile. d'Herelle himself considers this discovery to be identical with his own.

In 1899 Gameleia describes the so-called chromatolysis. This has so many properties in common with d'Herelles phenomenon

that it is difficult to reject the thought that they are identical. The reaction in the substratum must be alkaline or neutral and the bacteria must be undamaged. The lysis is inhibited by some substances, promoted by others. Among the latter casein must be mentioned and especially the glutaminic acid contained therein. If this is allowed to influence the bacteria »it becomes still more virulent« against fresh bacteria i. e. still more suitable for the dissolution of fresh bacteria. By precipitating the »chromatolysed« bacterial cultures in acetic acid and dissolving the precipitation in ammonia, a bacteriolysin is obtained which not alone promotes chromatolysis, by which is meant a partial lysis of the bacteria with a residue of stainable granules, but dissolves them completely in the course of 6 to 12 hours. From the culture dissolved in this manner the lysin can again be precipitated ad infinitum. The lysins obtained are specific inasmuch as they can only dissolve the species of bacteria used in their production. Of these the following are mentioned: Anthrax, cholera, typhoid, pyocyaneus and tubercle bacilli. The bacterial lysins destroy the bacteria in animal organism, to the cells of which they are not toxic, hence Gameleia proposes their being used for therapeutic purposes.

The phenomenon described by Twort in 1915 shews likewise many points of similarity to that of d'Herelle and they may be regarded as identical. d'Herelle himself, however, is not of the opinion that this is the case and supports this belief upon the fact the 2 lysins possess a varying resistance towards heat. After it having been ascertained that different d'Herelle lysins have a greatly varying thermal death-point this objection must be considered as scarcely tenable.

The bacteria-inhibiting and germicidal substances described by Ejkmann, Conradi and Kurpjuweit must, on the contrary, be considered as differing from d'Herelle's. (Otto and Munther).

Of the principal problems under which a review of the literature might be arranged the question as to the nature of the lysin takes precedence. Is it a virus, a ferment, a hereditary disease in the bacteria or a factor that does not come under hitherto known and described phenomena.

In connection with this, questions arise such as the relation of the lysins to physical, chemical and mechanical influences.

Furthermore the question as to the plurality or unicity of the lysins. Is there but one lysin, and that, capable of assuming various properties, or do several lysins exist, each characterized by different properties, as for instance, their capacity to dissolve different species of bacteria.

Again there is the question as to how the process of dissolution itself takes place; its velocity; its variation in varying circumstances and the more or less complete dissolution of the bacteria. In close connection herewith there is the question regarding the susceptibility and resistance of the bacteria against the lysins with the formation of the peculiar and numerous forms of resistant bacteria.

Affiliated with the question of the nature of the lysins, are also the researches regarding their serological peculiarities.

The question as to the occurrence of the lysins and the dissoluble species of bacteria is of a more isolated nature.

Finally there is a question the practical significance of which by far exceeds the more theoretical in the above-mentioned viz. the therapeutic application of the lysins.

An important condition for the satisfactory solution of these problems is to have the most accurate possible method for measuring the potency of the lysins.

THE NATURE OF THE LYSINS.

Before taking in hand a review of the researches that are of import in this connection, it would be practical to emphasize that by term "lysins", I, for my part, have taken no standpoint as to the nature of the phenomenon dealt with. It would perhaps be regarded as natural to employ the term "bacteriophage"; but as this in a high degree involves taking a definite stand as to the nature of the phenomenon and as it does not possess the same applicability as the word "lysin" I have chosen to employ the latter throughout this treatise. I have made some exceptions where the omission of the word "bacteriophage" might confuse the meaning.

To avoid any misunderstanding it must be remarked that d'He-

relle, who is of the opinion that the lytic capacity in the filtrates is due to an ultravisible virus, attributes it to the capacity of secreting lysins that consitute a link in the process of dissolution. When I use the term "lysin" it does not signify this, but it is used as a term for the whole phenomenon.

d'Herelle's fundamental researches have been described by me in an earlier publication, therefore I shall not enlarge upon them *). As is known he filtered suspensions of faeces from dysentery convalescents and discovered that the filtrates would dissolve dysentery bacilli. A small quantity of the dissolved culture was able to dissolve a fresh culture and thus the dissolution of fresh cultures could be continued in series in unlimited numbers.

d'Herelle presents the following theory: In the faeces of dysentery convalescents there is an ultravisible virus that can be filtered through porcelain. The virus that he calls bacteriophagum intestinale is corpuscular. It dissolves the bacteria by penetrating into them, multiplying in their interior and bursting the bacteria, thereby liberating a new generation of bacteriophages. If to a dysentery culture a small number of bacteriophages are added, these when the culture is dissolved will have multiplied in such a manner that the solution now contains many times the number of those originally added. A small quantity of the solution is therefore capable of dissolving a young culture and so on. The bacteriophages are compulsary parasites on the bacteria, being only capable of multiplying on living bacteria and on no other substratum. On the basis of this d'Herelle attributes to the bacteriophages a decisive significance with regard to the capacity of the higher organism to resist, perhaps to overcome infection by bacteria capable of being dissolved by bacteriophages.

The theories subsequently advanced to explain d'Herelles phenomenon are fairly numerous, but frequently mere modifications of those already advanced. In the following I shall only refer to theories that deviate essentially from each other. Later I shall show what speaks for the correctness of each of these in particular.

Kabeshima was of the opinion that the process was due to a ferment. All bacteria contain a pro-ferment (against themselves).

*) Hospitalstidende 1921.

When bacteria infect an animal organism, some substance or other is formed that transforms the pro-ferments into a ferment that thereupon dissolves the bacteria. Hereby fresh ferment is formed. By the action of thus formed ferment, or as he in a later communication calls it, autolysin (resp. proautolysin), on the young bacteria their proautolysin is transformed into autolysin and they are dissolved.

Bordet and Ciuca advance a theory that on certain points resembles Kabeshima's. They explain the phenomenon without assuming the presence of a virus, as a co-operation of external causes and heredity. They take for granted that an external cause in relation to the bacteria, a link in the self-defence of the organism, acts upon these. Hereby they are supplied with an increased autolytic capacity that by heredity they are able to hand down to new generations. The same substance that originally caused the increased autolytic capacity of the bacteria, they, in their new state, will be able to secrete and thereby, besides producing more easily autolysible individuals, will also be able to transform young individuals.

Bails theory has the same point of departure as the latter, for he takes for granted an action originally derived from the organism upon the bacteria. He gives no further explanation, but expresses that it possibly originates from the leucocytes. He stated that from the bacteria so-called "splitter" were separated, minute particles of bacteria capable of continuing to exist as independent individuals. These "splitter", he said, possess the capacity from fresh bacteria, while these were developing, to liberate new "splitter". Likewise under suitable conditions they were said to be able to develop into bacteria of the same species as those from which they originated. As a parallel to this he mentions the researches of Weils and Felix on the various types of exanthematic typhus proteus.

In subsequent communications Bail enlarges upon and supplements this theory by investigating the mechanism during the "splitter" formation. Here he makes use of conclusions ex analogia from the manner in which higher organisms are propagated. He attributes to the bacteria a nuclear substance consisting of chromosomes that, each in particular, represents hereditary properties in the bacteria. Some chromosomes represent destructive properties and others con-

structive. The chromosomes that collectively possess all the hereditary properties of the bacteria are more or less solidly united.

If we now imagine that a chromosome furnished with destructive properties is detached from the bacterium and that this chromosome is capable only of dissolving other bacteria when these are about to multiply we have then the bacteriophage. After a chromosome has become detached the remainder of the bacteria continues to live and propagate fresh bacteria all of which lack the detached chromosome. Being deficient of this chromosome results in their not being capable of being dissolved by chromosomes similar to those that are detached from them, but possibly by others. This explains why a bacteria capable of resisting one lysin can be dissolved by others.

In this manner the genesis of bacteriophages and the resistant bacteria is explained. It remains to elucidate why some bacteria are dissolved and some become resistant bacteria. If the chromosome detached is a vital part of the bacterium, the latter will quickly disintegrate; should it be merely an insignificant particle of the nuclear substance of the bacterium, the remainder will continue to live as a resistant bacterium. By means of this theory the plurality of the bacteriophages and the capacity of individual bacteriophages to dissolve bacteria of different species are explained, for the concordance between individual chromosomes in the various bacteria presents unlimited possibilities.

The theories opens up possibilities for the study of the component parts of the bacteria, as in the bacteriophages there is a means of separating them. Let us, for instance, imagine that a bacterium is exposed to a certain bacteriophage and thereupon successively to others, we have here the possibility of determining the composition of one bacterium as compared with that of another subjected to the same procedure. Bail has experimented in this direction and has demonstrated that the breaking down of Shiga bacilli is a far simpler process than that of Flexner bacilli.

As regards the multiplication of the bacteriophages Bail agrees with the aforementioned theory on the point that there exists no multiplication in the common sense of the word, but that it is merely a question of a concentration resultant upon the process of destruction.

On the point as to whether it is a question of a virus or a fer-

ment Bail adheres to the opinion that it is neither, but a completely new phenomenon for which we have no designation. As do several authors Bail lays no main stress upon the dissolution itself, but regards it as a link in both artificial and natural bacterial mutations.

Bail himself emphasizes that his theory is only a theory, but being in agreement with all the phenomena discovered by others he himself regard it as a good working basis.

Whether we can agree with him or not on this point is a matter of opinion. The simplest possible theory is to be preferred as a working basis and it appears to me that d'Herelle's in this respect possesses advantages over Bails and at the same time, as d'Herelle has emphasized time after time, it has not been refuted on any single point.

A number of writers contend that the lysins are ferments. Borchardt thus opines that it is trypsin. Combiesco produces lysins from trypsin and enterokinasis; Costa Cruz compares the action of the trypsin and its genesis with that of the thrombin; Davison is of the opinion that it is an enzyme but not trypsin. Ehrenberg and Løventhal opine that it is a proteolytic ferment; Pico produces lysins from trypsin and pancreatin.

Doerr, Eastwood, Schnabel and Yasaki advance the theory that it is due to increased metabolism in the bacteria, others designate it as a disease of the bacteria (Preitz).

Salimbeni contended, at an early juncture, that the lysin was a fungus that under certain circumstances could be observed microcroscopically.

As already mentioned besides the experiments given here to elucidate the nature of the phenomenon, there are numerous others. To take a stand as to which is the most probable is no easy matter. The main question will necessarily be whether we are faced with an organism that fulfills the claims generally laid down in respect to the designation "life" or whether we are confronted with something that must be classified in "the ranks of the inanimate". Here we must clearly understand that the lysins may easily be products generated by a living organism, for instance, bacteria or the infected organism, but in such cases it would be justifiable to classify them in the latter of the two categories mentioned.

Opinions differ as regards the claims to be made as to the function producing the phenomenon in order to apply the designation "life". Claims such as the capacity to multiply, metabolism, and a certain sensitiveness towards exterior influence are not absolutely applicable in this connection. In respect to the capacity to multiply we can, when it is a question of the invisible, scarcely determine whether this exists or whether it is a question of an increase in quantity independent of multiplication. As regards the possible metabolism a similar consideration holds good. As to the claim to susceptibility to outer influences, this is based upon the fact that though this analogies might be drawn as to the susceptibility of other living organisms; here, however, the possibility of life cannot be excluded even if the organism in question could withstand influences not otherwise compatible with life. As long as the lysins are invisible we must be content with probabilities. In the following we shall examine which of the possibilities mentioned is rendered most probable from the investigations made.

The effects of the lysin on solid substrata are localized. Should a mixture of lysin and bacteria be spread the characteristic plaques will be obtained in the culture. A soluble substance should give a uniform effect throughout the whole culture. This, that originally was one of the main points in d'Herelles theory is not unequivocal, for the lysin might be connected with corpuscular elements that in themselves possessed no lytic capacity. These elements might be bacteria (Bordet, Ciuca, Kabeshima, Watanabe and others) or particles of decomposed bacteria (Otto and Winkler). The localized effect might depend upon only a certain number of the bacteria being susceptible. Thus there is no dearth of explanations that the effects of the lysins may be localized, even if it be insoluble. It has been attempted to determine this question by means of dilution experiments which will be mentioned later.

As already stated the capacity to multiply of the lysin has not been finally determined. A catalyser, a ferment or an activater of a pro-ferment can produce the same effects that by others are interpreted as multiplication.

To prove that the lysins originate from the bacteria it is emphasized that frequently lysins are found in old bacterial cultures

without these ever having being in touch with lysins (Arkwright, Bail, Bordet and Ciuca, Flu, Gildemeister, Jøtten, Kuttner, Lisbonne and Carrere, Otto and Munter, Weinberg and Aznar). Against this, however. is justifiably objected that the bacteria might be infected from the primary isolation and that the virus was conveyed throughout all seedings without shewing up (d'Herelle). That this is possible has been demonstrated by Flu. That lysins are so frequently to be found in old cultures consequently denotes nothing as to their nature.

Bordet and Ciuca supported their theory by an experiment in which they injected B. coli into the peritonaeum of a guinea pig and in the exudate formed found lysin. The experiment has been repeated by numerous writers, but in a few cases only has been reproduced. This seems to be explainable by the aforementioned circumstance, to wit, that in the culture used by Bordet and Ciuca lysin was already present. This is also made probable by the fact that Bordet and Ciuca themselves have produced lysin from old cultures and that the culture used is identical with that used in the guinea pig experiment. Bordet and Ciuca attribute to the leucocytes considerable significance in the formation of lysins. In the peritoneal exudate of guinea pigs as also in the feces of convalescents after intestinal diseases they found numerous leucocytes. Bordet and Ciuca are of the opinion that the lysin in both cases is probably due to the leucocytes. This assumption is, however, somewhat invalidated by the experiments of Marcuse, in which he found lysins in the urine of rabbits in the bladder of which B. coli was injected. In the samples of urine no leucocytes were found. Also on examining the leucocytes of normal or immunized animals no lysins were found. Pico was successful, however, in producing lysins from leucocytes.

As a matter speaking in behalf of the virus nature of the lysins, their adaptability to various bacteria is emphasized, for it is contended that a dead agent cannot possess such. d'Herelle and others are of the opinion that they have demonstrated that by cultivating lysins in the presence of bacteria they are incapable of dissolving, they can make them active against these. According to Bail's researches this, however, is incorrect. He contends that it is due to

the majority of lysins being composite lysins (mischbacteriophagen), consisting of several component-lysins (Teilbacteriophagen). Some of these are so few and weak that they do not manifest themselves in the mixture, but may be cultivated when the lysin is brought into contact with bacteria to which the weak lysins possess a special affinity. Lysins in pure culture, i. e. those that cannot further be divided into component lysins, are according to Bail only capable of dissolving a certain number of bacteria and by no means can be induced to dissolve more. Bail's researches are extremely careful and comprehensive. They appear in a satisfactory manner to elucidate that which d'Herelle and many others have presumed to be a capacity for adaption in lysins, and likewise they also explain Maisin's discovery that a lysin can be changed is such a manner that from being active against 2 species of bacteria it becomes active against one of these only.

The adaptability of lysins to chemical and physical influences, that also was included in the discussion as to their nature, will be treated later.

Many writers endeavour to explain d'Herelle's phenomenon as the effect of a ferment. Kabeshima and Bordet included ferments as a feature in their theories, but were of the opinion that there was something else, something according to Kabeshima that could activate a proferment contained in the bacteria, and according to Bordet and Ciuca, a substance originating from the infected organism (leucocytes) in connection with an hereditary disposition in the bacteria.

The assumption that the lysins were ferments arose, inter alia, through the observation that they were frequently found in the alimentary canal. Some, therefore, have endeavoured to identify them with one or several of the ferments of the alimentary canal; others consider them to be independent ferments.

The most fundamental researches on this point are Borchardt's. In a fine work, the technique and results of which will subsequently be mentioned, he examines all the secretions of the alimentary canal and also extracts from various organs and finds that a mixture of extract of pancreas and extract of duodenal mucous membrane cause a dissolution of bacteria that is to be identified with that discovered by

d'Herelle. Consequently he is of the opinion that the lysins probably are identical with trypsin "in statu nascendi". Borchardt was not successful in producing lysins from existing mercantile preparations of trypsin or pancreatin, other writers were, however, successful in doing so (Pico, Combiesco and others).

Keller's investigations, however, tell against the lysins being identical with any of the commonly known ferments of the animal organism. Justifiably he claims that in order to determine such an identity, everywhere where lysins are found properties must be demonstrated, that agree with those of the ferment, and everywhere where the ferment is found in an effective form lysin must be demonstrable. By means of comprehensive researches he finds that neither of these conditions are complied with. If the lysins are ferments they must consequently be independent ferments and this view is shared by numerous other writers.

Pico who produces lysins from pancreatin thus considers that the pancreatin, during cultivation in series, disappears whereas the lysin increases in strength. Seiffert opines that lysins exercise an increased action when trypsin is present, whilst other substances have an inhibitory effect. In this connection it must be mentioned that Seiffert is of the opinion that all bacteria produce autolysins and that these are inhibited by some substances and promoted by others. The fact that lysins do not, as a rule, make themselves known is due to their being destroyed just as quickly as they are formed. Jøtten likewise produces lysin from pancreatin and he is the only writer who has been successful in further cultivating lysin upon killed bacteria.

Otto and Munter also adhere to the theory that lysins are ferments, whereas Prausnitz, on the basis of comprehensible filtration and dialysis experiments, finds that the lysins differ from the commonly known ferments, likewise in their relation to a number of chemical substances they differ widely from ferments. From the researches as to the adaptability of the lysins to antilytic sera, he is most inclined to regard d'Herelles theory as being correct.

Consequently it is scarcely probable that the lysins are identical with hitherto known ferments and, as yet, it is not proved that they, on the whole, are ferments.

Doerr, Arkwright, Eastwood, Reichert and Yasaki contend that d'Herelle's phenomenon is a result of an increased meta-

bolism in the bacteria. Doerr especially supports this with significant arguments. All authors excepting one (Jøtten) are agreed that the lysins only multiply in the presence of living bacteria and that it is not sufficient that the bacteria be living, but also that they must multiply. On this basis Doerr opines that the lysins can well be regarded as a result of increased vitality and increased decay. That a decaying process occurs he does not deny, but he regards this as a result of increased metabolism in the bacteria. The result of the increased vitality is that the bacteria produce lysin, become lysinogenic and lysin resistant. This is made probable by the fact that the bacteria may acquire these properties without any of them being dissolved, which occurs when they are brought into contact with lysin in mucilaginous substrata; many authors have also observed that lysogenic and lysin resistant bacteria occur naturally without connection with lysin.

Doerr compares these circumstances with those connected with cancer and considers that they are analogous; an external irritant causes augmented growth inertia and increased decay, the question involved is merely what the irritant is and how it acts. The view that autolysins are not involved Doerr furthermore supports by the fact that lysins have not been discovered against those bacteria that empirically are inclined to autolysis (pneumococci, meningococci), whereas bacteria that empirically are less inclined to autolysis, (typhoid-coli group and many others) are the especial field of action of the lysins. Moreover he finds that the dissolution of the bacteria occurs more slowly the more ill-nourished the bacteria are. Faced by a large surplus of bacteria the lysin is inactive, the irritant not being sufficient. The cause that the dissolution occurs when the bacteria multiplies is that the multiplication is conditioned by a membrane lesion and that young bacteria have a more vulnerable membrane, both of these factors making them more susceptible to external irritants.

He poses the question as to whether d'Herelle's phenomenon depends upon a change in the metabolism of the bacteria, artificially brought about and whether the phenomenon, on the whole, occurs naturally. In order to decide this point he examines chicken embryos and 'ex ovo' sterile chickens for lysin. Mammals cannot be used as the lysins might be conveyed through the placenta. Intestinal extract from chicken embryos and sterile chickens never produced lysins,

this was also the case with sterilely kept and fed chickens 8 days old. In these latter lysin was not present in the excrements until after a lapse of 15 days and the lysins found acted only upon bacteria that (in spite of the sterility) were found in the chickens, but on no other strains. The investigations do not decide the question, but in themselves they are very interesting, shewing, as they do, that lysins may be formed of bacteria alone, in collaboration with the animal organism.

This theory of Doerr's that is based upon metabolic changes in the bacteria must not be confused with d'Herelle's that attributes a metabolism to the very bacteriophages as secrete the lysins.

Finally Wertemann's researches as to the excretory circumstances of the lysins in warm and cold blooded animals. He finds that the lysins are excreted in a manner that deviates considerably from that in which chicken-pest virus is excreted, whereas their excreation resembles those found in heterogeneous albumins. He is therefore not of the opinion that the substance in question is an ultravisible virus.

UNICITY-PLURALITY.

By the unicity of the lysins is understood that all lysins are modifications of one and the same fundamental lysin. These modifications are characterized by their capacity to dissolve bacteria of various descriptions; the size of the plaques they form on solid substrata; their serological behaviour; their resistance against heat and their relations in various other directions.

By the plurality of the lysins we understand that various different lysins are to be found that cannot be traced back to a single fundamental lysin, but that each in particular is characterized by its relation in the directions mentioned.

d'Herelle argues the unicity of the lysins from the following observations:

The majority of lysins are non-specific inasmuch as they dissolve bacteria of widely differing species. One lysin dissolves, for instance, coli and dysentery bacilli, but at the same time also staphylococci. Should we cultivate this lysin further for a long period, for instance

on coli bacilli, it retains the capacity of dissolving dysentery bacilli and staphylococci. If we find a lysin that, for instance, only dissolves staphylococci, it is possible to impart to it the capacity also of dissolving other bacteria, e. g. dysentery bacteria by cultivating it in a mixture of dysentery bacilli and staphylococci. If lysin is intravenously injected into the experimental animal we obtain an immune serum. This serum — the antilytic serum — can neutralize the lysin an gives complement fixation with the lysin as antigen. The complement fixing antibody in such sera is non-specific, inasmuch as it gives complement fixation with all lysins no matter how different in other ways they may be.

From other sources significant arguments as to the plurality of the lysins have been advanced. These that are based upon very extensive researches, carried out with great care and patience, are in virtue of this very convincing.

The authors who in this connection should be mentioned are Brynoghe, Appelmans, de Kruif together with Bail and his collaborators, Watanabe, Matsomuto and Yasaki. From these latter there exist a number of works characterized by their wealth of details and in which no trouble has been spared to solve these problems.

The questions treated by all these authors are almost identical, and as the results also in the main agree, they will here be mentioned collectively.

That which originally caused them to open their eyes to the fact that there existed several lysins was that by seeding on solid substrata, plaques were found of greatly varying sizes on the same plate. Should the lysins now be cultivated further from plaques of varying sizes it was discovered that a lysin cultivated from a small plaque frequently gave small plaques on reseeding, and a lysin cultivated from large plaques on being re-seeded gave large plaques.

Thus a naturally occurring lysin was easily distinguishable from among several lysins by means of the size of the plaques. Thereupon it was obviously suggested to examine whether these lysins also in other respects shewed differences. This to a very great extent proved to be the case and the result of the researches was that a lysin cannot be regarded as pure until it no longer can be divided into lysins possessing various properties.

The properties that permitted of the classification of elementary lysins or to employ Bail's designation "Teilbacteriophagen", eventually proved to be fairly numerous and it is especially Bail and his collaborators who have distinguished themselves by systematic researches in this domain.

As especially practicable for such type determination of lysins the following methods will here be given;

1) Classification by cultivating on solid substrata. If some of a mixture of a naturally occuring lysin and dissoluble bacteria are spread on solid substratum, plaques of varying sizes will frequently be found. As already mentioned these plaques on re-cultivation often give plaques of the same size. Frequently we shall find that during the first passages they split up, so that e. g. a lysin cultivated from a large plaque gives both large and small plaques. If the re-cultivation is continued it will, however, be possible to obtain lysins that at all other seedings produce plaques of the same size. The most difficult to cultivate (pure) are lysins with large plaques owing to the fact that they through several passages are inclined to split up into lysins with small plaques.

It is, however, necessary to be somewhat reserved as to the practicability of this method because the size of the plaques depends upon various extrinsic circumstances, such as, the moisture of the plates, the growth-velocity of the bacteria (the composition of the substratum), the density of seeding and various other conditions. The method is, however, indispensible as an introduction to a methodical research.

2) Classification by cultivating in fluid substrata.

It is especially the appearance of the culture during dissolution that is of significance. One lysin completely clears the culture, another produces a limpid fluid with a precipitation, a third gives no precipitation, but a fine flaky growth throughout the whole culture etc.

3) Classification by velocity of multiplication.

This is determined by taking samples during dissolution, killing the bacteria by heat and determining the number of lysin units per c. c. in the sample taken.

4) Classification by the field of the activity of the lysin.

a. The lysin is tested against a large number of various species and strains of bacteria.

b. The lysin is tested against a series of bacterial species and strains that are resistant against other lysins.

That this method (b) is practicable is founded on the interesting observation that a bacterium that by being cultivated together with a lysin has become resistant against it, may well be susceptible to another lysin. In fact it may even happen that just by becoming resistant against one lysin it becomes susceptible to another. As an example may be given a strain of typhoid A, that can be dissolved by a lysin a,—and a lysin b that cannot dissolve A. If A is cultivated together with a, a culture resistant against a is obtained, viz. resistant culture A'. Whilst A could not be dissolved by b, this is the case with A'. If we test in this manner a large number of strains and at the same time together with the corresponding resistant strains, there would be an unlimited number of possibilities for determining the inter-dependency of the two lysins.

5. Classification by serological differences.

If we inject a mixed lysin into an experimental animal an antilytic serum is obtained, i. e. a serum capable of counteracting the effect of the lysin injected. If the antilytic serum is tested against the individual lysins in the mixed lysin, it is demonstrated that the lysins are also neutralized singly by the antilytic serum. If we inject a lysin that cannot by means of any of the methods mentioned be separated into various lysins, an antilytic serum is obtained that only neutralizes the lysin or lysins employed that, in other respects too, prove to be identical with it.

In respect to the practicability of the complement fixing reactions for the separation of lysins opinions differ. d'Herelle opines, as already mentioned, that every antilytic serum produces complement fixation with every lysin. Costa Cruz on the contrary, is of the opinion that complement fixation is specific in the individual lysins, and Gratia and Jaumain demonstrate that the complement fixing antibody common to all lysins is not due to the lysins themselves.

6) Classification through the relation of the lysins to physical and chemical influences.

The various elementary lysins possess different thermal death-

points and varying resistant powers to the influence of chemical substances.

Thus there is the greatest probability of the plurality of lysins. One point that demands further elucidation before taking a definite standpoint is the fact emphasized by d'Herelle that a lysin which dissolves various species of bacteria (e. g. dysentery and staphylococci) may be further cultivated on the one throughout a practically unlimited number of passages without losing its lytic power against the other. If it were a question of various lysins it would be expected that the one would be eliminated through the continued dilution.

THE RESISTANCE AND SUSCEPTIBILITY OF THE BACTERIA.

If we test a given lysin against a larger number of bacterial species end strains we shall as a rule observe that it dissolves several of these. The bacterial species that one and the same lysin dissolves may be very heterogeneous e. g. typhoid bacilli and staphylococci. Within the same species a lysin seldom, however, dissolves all the strains examined. Of 10 typhoid strains 3 may be dissolved, whereas 7 cannot. In respect to dysentery, however, a lysin that dissolves one strain dissolves all other dysentery strains. This, however, is only the case with dysentery, the ratio with all other strains will be found to be the above-mentioned.

The strains that are not dissolved are consequently resistant or rather insusceptible. By resistant cultures in this connection as a rule something else is understood, viz. cultures with acquired resistance, in the sense that the original susceptible cultures by being cultivated together with a lysin have become resistant against it. The bacteria that have thus become resistant differ decisively in various ways from the susceptible cultures of the same strain. These conditions that were primarily demonstrated by d'Herelle and subsequently elaborated by Bordet and Ciuca have been thoroughly tested by numerous writers and in most cases the results agree.

The respects in which the resistant cultures differ from the original are the following: Morphological, cultural and serological, also

in regard to their mobility, their lysogenity and their relations to other lysins.

In the innumerable forms of resistant bacteria described in the literature these variations differ to such an extent that it is difficult to determine definite groups. Gratia thus describes 11 different variations within the same strain. Here it is not my intention to give a detailed description of all these forms, but merely in general to account for the main points.

Morphologically the resistant bacteria as a rule differ considerably from the susceptible. Frequently large bodies are found (2—3 times the size of the original bacteria) that, so to speak, are swollen. This and the long thick filiform are those most often observed microscopically in the resistant colonies.

The appearance of the resistant colonies likewise varies considerably within the same strain. Large flat opalescent, at times mother-of-pearl like, colonies with a definite circular margin, the consistency of which is solid, and, as it were, granular. Circular, raised very domed, and fairly transparent colonies may be found that impress one as consisting only of mucus in that they can be drawn out threadwise. A peculiar form is the subsequently described "nibbled" and "moth-eaten" colonies. Finally small whitish solid colonies may be found consisting as a rule of coccoid bacteria.

In fluid cultures the resistant bacteria will almost always be found growing as a more or less voluminous precipitation that only with difficulty can be shaken into a homogeneous emulsion. The resistant bacteria are, as a rule, very mobile, even if the bacteria of which they are modifications are immobile. Gratia has thus separated resistant and susceptible bacteria by cultivating them in a semi-fluid substratum placed in a U tube in the one branch of which is seeded a mixture of both.

The resistant bacteria are generally more toxic and to a greater extent pathogenic to experimental animals than the susceptible bacteria of same strain.

Regarding the serological conditions of the resistant bacteria their agglutinability is as a rule either considerably reduced or entirely checked by an immune serum produced from susceptible bacteria of the same strain. Whether they are also inagglutinable in respect to

a serum produced by injection of the resistant bacteria themselves, I have not seen elucidated anywhere.

The resistant bacteria are frequently lysinogenic i. e. that they produce in a susceptible culture the same lysis as the lysin itself. This is always the case with the bacteria in the "nibbled" colonies.

As a matter of course the resistant bacteria cannot be dissolved by the lysin that has produced their resistance, but this, as formerly mentioned, does not prevent their being dissolved by other lysins. It may even happen that bacteria that are insusceptible to one lysin by becoming resistant to another may become resistant to the former.

This circumstance which is very peculiar involuntarily calls to our minds the question of what causes the resistance of the bacteria. In the case of some resistant bacteria there is an explanation that appears to be quite feasible. Here I refer to bacteria that form very mucous colonies. As stated elsewhere mucilage acts inhibitorily on the lysin. Bail has demonstrated that the mucilage washed off such bacteria also has an inhibiting effect. As all resistant bacteria are not mucilaginous the resistance must also be due to other circumstances that are certainly of a more profound nature. Researches in this domain are very rare. While mentioning Bail's theory it was stated how he explained these circumstances by presuming that particles of the bacteria under the influence of the lysin became detached, whereby the properties of the bacteria were altered. The experimental basis of this theory leaves so much to be desired that before accepting it we must investigate whether there are other explanations more probable. Hereby the question of the variation of the bacteria in general arises. Many authors have found variations of diffent bacteria that correspond accurately to the bacteria resistant against the lysins, without the strains examined having ever been in contact with lysins. Gildemeister, long before d'Herelle discovered the lysins, has described the so-called "flatter-forms" that correspond accurately to the colonies of the resistant bacteria. Subsequently similar variations without demonstrable lysin were discovered by Bergstrand, Flu, Jansen and Wolff, Lisbonne and Carrere, Otto and Munter, Seiffert and Zdansky. In this connection we must, however, remember the ubiquity of the lysins, they having been demonstrated in almost all naturally

occurring media. d'Herelle's contention that lysins when found in cultures that have never been in contact with lysins must be due to the cultures having been infected with the lysin, ever since the first isolation of the bacteria, without this being noticed, likewise applies in this case. Variations occuring naturally that are identical with resistant bacteria may well be presumed to be resistant bacteria produced by lysin that has escaped observation.

Here we encounter one of the main questions within lysin research. The problem may briefly be formulated as follows: Are naturally occurring bacterial variations usually conditioned by the presence of lysins or are the lysins a function of naturally occurring bacterial variation? Concerning this problem there exist some few investigations. As already mentioned it has been supposed that the transmissible lysis was a "laboratory phenomenon". In support of this it may be mentioned that Flu discovered a strain of dysentery that from the very first culture and throughout 300 passages was resistant to all lysins tested, but was not lysogenic. By destroying the bacteria with sulphate of natrium he liberated lysin. Zdansky's interesting researches on the conditions in nature for lysin may likewise be mentioned. He comes to the conclusion that these are fairly poor. The frequent presence of colloids, the unfavourable conditions of temperature and reaction on the whole, together with quantitative disproportion only allows an inconsiderable spread of the lysins. He draws comparisons between the importance of the antibodies in the laboratory and in nature on the one hand, and the importance of the lysins under identical circumstances, on the other. He regards the resistant bacteria as saprophytic forms of the virulent bacteria and this he supports by means of researches regarding the occurrence of both forms. He finds that the percentage of susceptible bacteria is far higher in the vicinity of the source of contamination than that of the resistant, whereas the circumstances differ in the opposite direction the farther off we are.

At the presence juncture we can get no further than an acknowledgement, that by means of the lysins, bacterial variations may be produced that are absolutely identical to variations of the same bacteria occurring naturally. It remains to future research to determine to what extent any connection between these two phenomena exists.

One point that has given occasion to a great deal of discussion is the question regarding to what extent the resistant bacteria are, in advance, presence in an otherwise susceptible culture or whether all the bacteria in the culture are uniform and do not split up into bacteria, some dissoluble some indissoluble, until they are under the influence of the lysin. Gratia and many others are of the opinion that the culture must consist of two sorts; this opinion is founded on the fact that in cultures not exposed to lysin, resistant bacteria may occur that in everything are identical with lysin produced resistant bacteria. This question can scarcely be further elucidated than I have done in my researches viz. by microscopically determining that some of the bacteria under the action of the lysin are dissolved and that others grow simultaneously into well characterized colonies. Whether this latter depends upon some of the bacteria in advance possessing properties differing to those possessed by other or whether it is the lysin or irrevalent circumstances that cause the separation, may possibly be decided by a numerical quantitative examination of all the bacteria in the culture. The phenomenon might even exclusively depend upon the possibilities of meetings between bacteria and lysin units.

A question of great interest is the stability of the variations. That this under homogenous external conditions is considerable seems to be established. Certainly it is observed that a resistant bacteria "spontaneously" changes into the susceptible form and the reverse may also occur, but as a rule, the resistant as well as the susceptible retain their properties throughout innumerable passages.

The resistant variations can be restored to their original susceptible form by cultivating them on substrata containing antilytic serum. At times normal serum may havn the same effect. (Bordet and Ciuca, Bail and others). As far as I know, no researches have been made as to the cause of this circumstance.

The importance of the resistant bacteria for the determination of type of lysins has already been mentioned.

Numerous researches regarding the therapeutic practicability of the lysins have been made, but of this later. The resistant bacteria occur here as a difficult complication. Their significance in this connection will be mentioned together with lysin therapy.

THE INFLUENCE OF CHEMICAL AND PHYSICAL CONDITIONS ON THE LYSINS.

The vagueness existing in this domain is probably due to the fact that the lysins on this point, as on so many others, show pronounced differences. When to this is added that the experimental conditions employed were frequently very deficiently defined, it will be readily understood why the results are so varying.

Appelmans, Prausnitz and Zoeller agree that the lysins are very susceptible to radiation with ultra-violet rays. According to Brutsaert they cannot bear radiation with radium.

With regard to the effect of soluble chemical substances upon the lysins, at the present juncture it is scarcely possible to determine definite proportions, the statements being, as a rule, so varying that they are not reconcilable.

Brofenbrenner and Korb thus find that the lysins are destroyed by 95 % alcohol at room temperature in 15 minutes and that lysin cannot be demonstrated in the precipitation. Other authors, such as Kuttner, Proca, Kabeshima, d'Herelle and others find, on the contrary, that the lysins withstand concentrated alcohol well and that they are precipitated by it.

According to the majority of authors lysins can withstand the action of ether and chloroform (d'Herelle, Kabeshima, Bablet, Prausnitz).

Fluoride of natrium can be tolerated in a concentration of 1 %, 3 % fluoride of natrium destroys the lysin in three days.

In higher concentrations glycerin acts destructively on the lysin. As demonstrated by d'Herelle and confirmed by several others, it is possible by increasing gradually the concentration to attain that the lysin remains unaltered even in high concentrations (50—75 %), nay, it is even capable of multiplying in the presence of 60 % glycerin (d'Herelle).

Carbolic is tolerated relatively well. Otto and Munther state that lysin can multiply in the presence of 0.1 % carbolic.

Tetralin has a very fatal effect upon lysin, whereas it does not exercise any harmful effect on ferments.

Quinine chloride has a pronounced destructive effect upon the lysins (Brutsaert, Eliava and Pozerski, Watanabe).

Watanabe has investigated the effect of a number of disinfectants upon the lysins with the object of employing the various resistances towards such in the differentiation of the various lysins. He finds that a definite lysin always remains constant, but that the individual lysins present considerable variations. Generally speaking he is of the opinion that the lysins in their relation towards disinfectants rank between bacteria and bacterial spores.

The thermal death-point of the lysin is by many stated to be ca. 70° C. d'Herelle, Bordet and Ciuca state 60—65°. Other authors such as Hajos, Pico and many others contend that the lysins can stand a heating to 100° for several minutes. Here, as on many other points, opinions vary greatly. The reason for this may be sought partly in the fact that the lysins vary and partly because the experimental conditions of the individual authors have been different.

Doerr and Rose have examined the significance of the experimental conditions in the determination of the thermal death-point of the lysins. They find that the following factors are of decisive significance. The reaction, the concentration and age of the lysin; whether the destruction is carried out in open or closed vessel and the presence of various bacterial metabolic products in the lysin.

They find, thus, that the lysin is far more quickly destroyed in closed than in open vessels. If the lysin is heated diluted with bouillon it keeps much longer than when correspondingly diluted with destilled water. Diluted with destilled water the lysin is already destroyed at 37° in from 7—19 hours. If chloride of natrium is added to the water they keep considerably better, and better still in the presence of divalent cathions, e. g. $CaCl_2$.

From this will be seen the rôle played by the experimental conditions under which the heating is carried out. As so to speak none of the authors who have occupied themselves with the thermal death-point of the lysins have stated the experimental conditions with anything like accuracy, it would be idle to judge the question from the data on hand. In general, however, it may be said that the lysins as usually produced, i. e. in bouillon, have a thermal death-point

lying between 65° and 73° C. in one hour; the destruction, however, in the case of some, begins already at 50°, but this is the minimum.

The majority of lysins can endure desiccation even if they to some degree are weakened thereby (d'Herelle, Caplazi).

Olsen and Yasaki caused some sensation in 1923, by stating that the lysins were destillable. Numerous authors have thoroughly tested this, in the case of some under identical experimental conditions as Olsen and Yasaki, none, however, have been able to confirm this finding. Some have even, under the given experimental conditions, been able to demonstrate transmission of drops.

Similarly it caused a sensation when Putter and Vallen reported that by filtering ordinary laboratory bouillon through Berkefeld filters they could produce lysins. A closer examination of the filters shewed that they previously contained lysin. The majority agrees that filtration strengthens the lysins. How this happens has only to a very limited extent been the subject of research. Hajos is of the opinion that it is due to the fact that filtration removes inhibiting substances. That the filters, in spite of the fact that filtration strengthens the lysin, retain a quantity of it has been demonstrated by Keller and Seiffert. Seiffert considers the lysin to be a coctostable activator. If it be heated to 100° for one hour it becomes inactive, but after filtration it becomes active again. It is not, however, all filters that are capable of re-activating the lysin after it has been boiled, Such an activating of lysins is refuted by Doerr and Zdansky.

That the conditions under which the filtration is carried out are of decisive significance cannot be emphasized too much. Gildemeister has moreover demonstrated that this applies not only to the pressure under which the filtration is carried out, but also to the chemical and physical properties of the filtrate. In very alkaline solution nearly the whole quantity of lysin passes through whereas in a very acid solution next to nothing passes through the filter. In all filtration and dialysis experiments a quantitative examination of the amount of lysin should be made before and after the filtration or dialysis. This requirement, in the case of the majority of authors, has not been satisfactorily complied with.

To the question as to the extent to which the lysins are cap-

able of dialysing great interest attaches. Their capacity in this respect is employed partly to determine their size, partly to decide the nature of the lysins by comparison with the capacity to dialyse of other substances. Unfortunately, here it also applied that the experimental conditions make the experiments equivocal.

d'Herelle dialyses through collodion membranes and finds that both the lysins and also the albuminous substances in horse serum pass through the membrane. The experiments from other quarters have been designated as worthless as the albuminous substances in horse serum are not capable of dialysing.

Prausnitz dialyses through Haensche membranes and finds in comparison with gelatine, haemoglobin, and collargol solutions that the lysin possesses a particle size of ca 20 μ/μ. Ferments such as pepsin, trypsin and invertasis passed through the densest membranes.

Ehrenberg, by dialysis experiments also through Haensche membranes arrives at totally different results.

Wolman dialyses through collodion membranes. He makes the test in the following manner: First he seeds the solution with bacteria on the one side of the membrane and applies the lysin to the solution on the other. The experiment is carried out at 37° and he finds that the lysins dialyses through, the bacteria being dissolved.

On the other hand he observes that the concentration of lysin, in the solution seeded with lysin alone, has also increased. He explains this by saying that bacterial metabolic products have diffused through the membrane and that owing to these the lysins have increased in strength. The lysins thus do not require living bacteria to propagate, the metabolic products of these latter being sufficient.

Doerr and Zdansky demonstrate that bacteria in 16 hours penetrate extraction containers (Schleicher and Schüll) covered with collodion, in 8 out of 10 experiments. In the 2 experiments in which the membranes were not penetrated, the lysin also did not pass through. The penetration of only one lysinogenic bacterium completely compromises the result and it may be presumed that this is the cause of the results of several of the authors mentioned. Wolmann's demonstration, especially, that the lysin concentration increases when on the one side of the membrane sensitive bacteria are present, must be presumed to be a penetration, all other authors ha-

ving found that the lysins cannot multiply on bacterial products of any description.

Taking this and other sources of error into consideration Doerr and Zdansky arrive at the result that the lysins cannot dialyse and and are therefore of the opinion that they must be classified or juxtaposited with colloid solutions of a biphasic system and not with genuine homogeneous solutions.

Involuntarily we are here led to a contemplation of d'Herelle's dilution experiments. To prove that the bacteriophage was a virus, d'Herelle made an experiment in which he diluted the culture of bacteriophage so considerably that in a given volume it was calculated to contain less that one bacteriophage. Thereupon, if he took 10 similar volumes and placed them each in its bacterial culture, he should get a dissolution in those cultures to which one volume containing a bacteriophage, had been added but not in the others. If the bacteriophage, on the other hand, was a soluble substance it would be divided into the same quantity in all 10 volumes and the effect should therefore be identical in all the 10 cultures. The former proved to be the case.

The question now is whether d'Herelle's reasoning is correct. He compares the phenomenon with a biological phenomenon on the one hand, and with a chemical on the other, and as his own phenomenon coincides with the biological he concludes it to be of a biological nature.

In a biological reaction it will always be the absolute volume that is decisive, in a chemical invariably the concentration. The difference, however, between the two reactions is only apparent, depending as it does, upon our not having sufficiently delicate reactions to demonstrate minimum volumes (a single molecule) of chemical substances. In the case of a molecular solution, this, just as well as a suspension of individuals, must be capable of so considerable a dilution that it, in a given volume, contains a single molecule. This, in contrast to a single individual capable of multiplying, cannot be demonstrated. In the two reactions there is but a quantitative difference. The comparison with the two reactions separately can therefore not be used in deciding whether a phenomenon is of a chemical or a biological nature.

That in other respects there is a difference in the lytic process and a chemical reaction none will doubt, the one factor in the former being a living organism viz. the bacteria. Respecting the nature of the other factor, nothing is known and that the lytic process takes a course like a biological reaction may easily be explained alone from the fact that the bacteria are alive.

Another question is whether, in the lytic process, it is the lysin concentration or the absolute lysin volume that is decisive wheter or not the process sets in.

Gratia and Kruif dilute a lysin very considerably. From this dilution they take 1111 c. c. divided as follows into 4 tubes.

a. 1000 c. c.
b. 100 c. c.
c. 10 c. c.
d. 1 c. c.

The whole quantity is, in advance seeded with so many sensitive bacteria that sufficient are present in each tube to assure the occurrence of the process of dissolution.

In a, b and c a dissolution takes place, but not in d. From this they conclude that it is not the concentration, but the absolute volume that is decisive.

The hypotheses of the experiment are, however, erroneous. In a rationally arranged test of this description should be taken.

a. once
b. 10 times
c. 100 —
d. 1000 —

Then possibly one would arrive at the result that a. produced lysis, b. lysis in 5, c. in 50 and d. in 500 cases. That the result, however, would be as stated cannot with certainty be assumed. One factor that probably would cause a difference is the »reaction possibilities« between bacteria and lysin units. It is not certain that 10 lysin units with 10 bacteria would invariably yield the same result. Besides the possibility that the lysin units may be unequally divided amongst the bacteria, there is the possibility that e. g. 5 of the lysin units meet with bacteria at a spot where they produce another effect

than dissolution e. g. that the bacteria become resistant, or that they are not in any way affected. Finally the bacteria are not always identical and this also may cause differences i the result. If now together with 10 lysin units there are not 10 but 1000 bacteria the possibility of a lysin unit attacking a bacterium at some vulnerable spot thereby causing its disintegration is 100 times greater. In order to decide what qantitative proportions causes the process to take place, a quantitative numerical analysis of these conditions is necessary.

The only author who has worked with this problem is Angerer who with another object in view ascertained that the fixation velocity between bacteria and lysin units takes place at a far greater speed than that dependant on their numbers and size.

THE COURSE OF THE PROCESS OF DISSOLUTION.

Whilst the lysins and their effects upon bacteria have each seperately been the subject of numerous researches by which much valuable information has been obtained, the actual course af the process of dissolution has only been closely investigated by very few authors. Doerr especially, and his collaborators Grüninger and Meuli. I, myself, took up, at an early juncture, this investigations and in the experimental part of this work the results will be given.

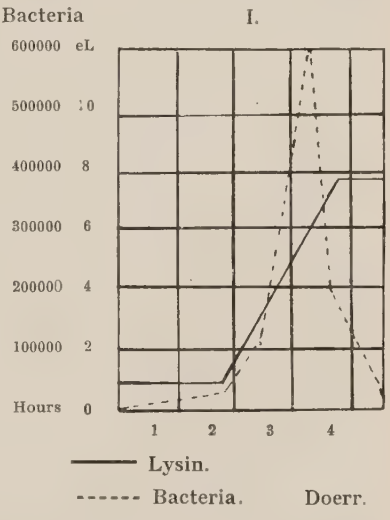
If a small quantity of lysin is added to a bacterial culture that is dissoluble by the lysin and the mixture placed at 37°, one will if working with lysin of medium potency, observe that the culture clears. When a certain degree of limpidness is attained the cloudiness again increases, disappearing subsequently either wholly or partially. Through many such periods one can attain to a complete clearing of the culture. This phenomenon, primarily observed by d'Herelle and later confirmed by all subsequent authors presents considerable interest. If seedings, on solid substrata, are made of equally large quantities of the mixture an counts are undertaken of the number of lysin plaques an bacterial colonies, it will be noted that gradually as the number of plaques increases the volume of bacteria decreases until finally no growth is obtained on the plate. Thereupon some isolated colonies again appear an these increase

in numbers in the subsequent seedings so that the plates are finally covered by a connected growth. Now plaques may again make an appearance and the whole development be repeated. What then is the signification of this undulating movement in the course of the process of dissolution? This question will be dealt with later. At this juncture mention will merely be made that Doerr and independantly of him, I, myself, have come to the conclusion that the fluctuations are not due to fluctuations in the lysin concentration, but fluctuations in the number of bacteria and their susceptibility to the action of the lysin. During the first phase of the process the lysin concentration increases until it reaches a maximum. This maximum is retained during the rest of the course of the process, whereas the number of bacteria capable of multiplying fluctuates.

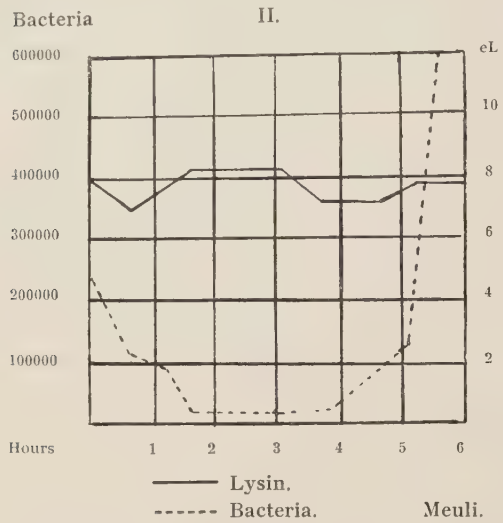
Several different factors determine the course of the process and of these the following will be mentioned:

The two main factors are lysin and bacteria. In addition to these the medium and the temperature at which the process takes place play a rôle. If these two factors are kept at optimum., the influence of the two main factors may be considered seperately.

It is natural primarily to note the importance of bacteria. It has previously been mentioned that these have to be alive. The method employed by Doerr and his collaborators to determine the number of bacteria is, in the main, identical with that used by me. It is of greatest interest to note what significance the initial concentration of the bacteria has. It is namely proved that this within extensive limits is a matter of indifference as to the course of the process and the final result. Meuli thus states that it may vary fra 100 to 1.000.000 bacteria pr. c. c. without any difference being demonstrable. If the initial concentration of the bacteria is kept uniform we may separately consider the significance of the primary concentration of the lysin.

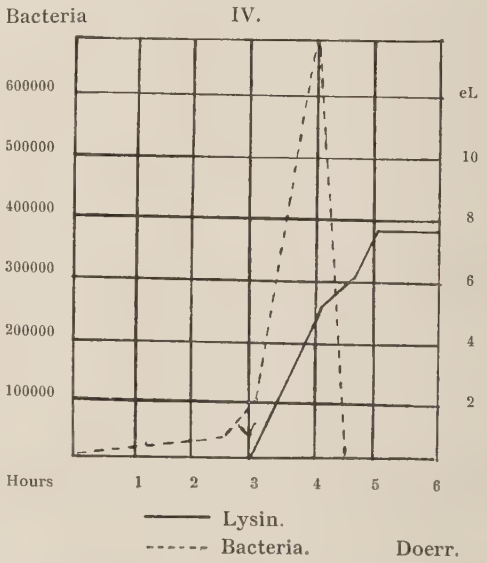


1) On observing the result with a minimum initial concentration, we see that both the lysin and the bacterial concentration remain unchanged for some time (1—2 hours), whereupon they begin to increase.



When the lysin concentration has reached its maximum which occurs as a rule in the course of about 2 hours, it remains constant, whereas the bacterial concentration decreases very rapidly, having in the course of 1 hour fallen to zero or thereabouts. (I).

2) If we now observe how the process develops when the initial concentration is at maximum, i. e. we seed a concentrated lysin with bacteria, it will be noted that the bacteria immediately and rapidly decrease in numbers, reaching zero in the course of 2 hours. The lysin concentration remains constant the while. (II).



3) All intermediate concentrations of the initial lysin may be coordinated in a steadily increasing series between these two extremes. In weak initial concentrations the bacteria will multiply as though no lysin were present, when the lysin concentration thereupon has attained a certain strength the bacterial concentration rapidly falls towards zero. The greater the initial lysin concentration becomes, the less the bacteria will multiply before the dissolution sets in.

From this it will be seen that the final result as regards lysin concentration is invariably the same. The curve of the bacterial

concentration varies all according to whether the primary lysin concentration is high or low. With regard to the bacterial concentration, in the final stage of the process one of two things may occur. Firstly it may be reduced to zero i. e. all the bacilli are killed and the bouillon remains sterile. Secondly, they may not reach zero and if this is the case they will, after a longer or shorter period again begin to increase. As to how frequently the one or the other occurs, or the reason for its occurring no researches have been undertaken. Personally, I have never experienced the former.

With regard to the dependency of the increase of the lysin upon the formation of the bacteria, the following experiments by Doerr and Grüninger demonstrate how closely the two factors are connected. From the above curve it will be seen that the increase of lysin corresponds exactly with the multiplication of the bacteria when both lysin and bacteria are present at the commencement of the experiment. What happens if the lysin is not added until the bacteria have multiplied for some time? What happens is, that the lysin curve immediately begins to rise and thereupon the course is just as if the lysin had been present from the very beginning (curve 4).

Otto and Munther repeated the experiment of Doerr and his collaborators. The result arrived at is, in the main, identical, but on one single point their results deviate from the aforementioned. They find, that the lysin concentration never attains its maximum before the bacterial concentration has commenced to fall. This would thus indicate that the lysin increase may well be due to the decay of the bacteria.

Thereupon we shall observe what rôle the initial concentration of bacteria plays. A factor for the first time observed by d'Herelle, is that a considerable surplus of bacteria may inhibit the lysis and that the lysin in such a mixture disappears. d'Herelle explains this by stating that the virus on account of its being in such a small quantity succumbs in the struggle against the bacteria.

If d'Herelle's virus theory is to be acknowledged this explanation must be considered as probable. Gratia explains the phenomenon by assuming an adsorption to bacteria followed by inactivation; it is, however, not quite understood, why an adsorption, that certainly in all cases may be presumed to take place, should act inhibitorily because there were more bacteria. Actually this phenomenon

lacks a satisfactorily explanation. Should an attempt be made to render one, it seems to me natural, first to examine whether it depends upon the fact that the considerable concentration of bacteria (large number of individuals in a small quantity nutritive solution) prevented a sufficiently vigorous multiplication. As the increase of lysin is dependent on the multiplication of the bacteria, this would absolutely explain why it failed to appear in this case.

An investigation as to the influence of the two other factors, temperature and the substratum still remains to be undertaken.

The optimum temperature of the process lies between 37° and 40°. If we drop under 37° the process is retarded until at a temperature of ca. 20° only an inconsiderable increase of the lysin is obtained. According to Doerr and Grüninger the lysis only occurs at lower temperatures than 20° when the lysin concentration is maximum. If a temperature be selected of above 44° no multiplication of the bacteria is recorded and the process does not set in. If we choose a temperature of 43° the interesting course is noted that the bacteria multiply, though somewhat more slowly than at 37°, but in the same degree, however. The lysin increase however does not take place at all and moreover, after a lapse of some few hours the lysin can no longer be demonstrated, in spite of the fact that a temperature of 43° has no effect upon the lysin alone. This experiment that has been carried out by Doerr and Grüninger shews that it is quite possible to have a multiplication of bacteria without this causing any rise in the lysin concentration.

Lepper's experiment on the course of the process of dissolution shews that an adsorption of the lysin to the bacteria takes place. Her technique for the determination of the surviving bacteria must be said to be unsuitable, spreading as she does merely a drop of the mixture-lysin-bacteria upon agar. Hereby she arrives at the result that in the course of 15 minutes 99% of the bacteria have been killed. The error is due to the fact that she transfers lysin together with the bacteria she wishes to count. When after incubation she desires to count the bacteria the majority have been dissolved by the lysin.

If we now consider the influence of the substratum upon the course of the process we discover very interesting factors.

As a matter of course the substratum must not contain substan-

ces that inhibit the lysis. As such may be mentioned all substances that hinder the multiplication of the bacteria. When this requirement is fulfilled the next question is what substances the substratum must contain to insure the course of the process.? Costa Cruz has found that without electrolytes there occurs no increase in the quantity of lysin. The action of the electrolytes does not depend upon their increasing the osmotic pressure, it being possible to add sugar without any lysis occurring. In the substrata containing no electrolytes the bacteria grow well, it is not therefore due to a want of bacterial multiplication that the lysis does not occur. Costa Cruz calls attention to the fact that substrata without electrolytes can rid the bacteria of lysin. The species of electrolytes is a matter of indifference. These observations have been confirmed in detail by Lisbonne and Carrere. Costa Cruz assumes on this basis to be able to say that both d'Herelle's and Bordet's theories, as to the nature of the lysins, are incorrect, for the bacteria — should they convey an hereditary capacity of dissolution — ought to retain it in substrata not containing electrolytes, and an ultravisible virus ought to thrive in such.

The substrata must furthermore contain sufficient nutritive matter for the bacteria. If these are not present a decrease in growth of the bacteria will result and thereby a reduced lysin increase. Bail in this connection has investigated the effect of formerly used bouillon and water agar.

Lepper has demonstrated that an increase of lysin can be obtained on synthetic substrata — naturally in the presence of bacteria. Her technique is, however, not quite perfect for which reason the results must be regarded with reserve.

The hydrogen ion concentration is of decisive significance in the course of the process of dissolution. This question is dealt with in the experimental part of this work. Here, as in so many other circumstances, it applies that the various lysins present great differences and the statements of p_H optimum vary consequently very much. Gratia states that it lies about $p_H = 8$ to 8.5. According to Elivia and Pozerski the lysins are not only inactive but become destroyed when the p_H is under 2.5 and above 8.4. Davison states the optimum to be about 8 to 8.2. Watanabe states that a 10 %

soda solution only paralyses the lysins and that a 20 % solution is necessary to destroy them. Otto and Munther state that with definite lysin they obtained effect only at a p_H of 6.5. Costa Cruz reports on a lysin the optimum of which lies at 5.1. Simultaneously he states that lysins in very acid bouillons are precipitated, in faintly acid bouillons they are also precipitated, but can again be brought into solution by neutralizing the bouillon. Scheideegger finds that one cannot state the effect of the reaction without simultaneously stating the temperature. At temperatures of between 20° and 37° the lysin can tolerate for several hours a reaction corresponding to p_H 4.5, whereas this reaction destroys the lysin completely in 2 hours at a temperature of 56°. He finds that the process of dissolution does not set in until at a reaction corresponding to p_H above 7.

A question that both practically and theoretically is of great importance in the study of lysins is that of the consistency of the substratum. It need not be emphasized that lysis proceeds in a different manner on solid substrata than in fluid, this being a well-established fact. The points of similarity do not, however, lack, and Doerr's assertion that the lysin plaques on the solid substrata are merely miniature reproductions of the test-tube experiments is perhaps the nearest approach to the matter.

A totally different question is how the process of dissolution takes place in solid substrata. At an early juncture in my experiments, I encountered this problem while endeavouring to work out a measuring method for lysins. The simplest method, I discovered, was to mix the lysin-bacteria mixture with fluid agar cooled to 40° and to compare the number of colonies occurring with that in another agar plate to which, in an identical manner, the same number of bacteria, but no lysin had been added. The difference in the number of colonies of bacteria would give the potency of the lysin. The experiment was absolutely unsuccessful; the lysin certainly had the characteristic effects on the surface of the agar, but in the agar itself it did not make its presence known, either in the number or appearance of the colonies. Thereupon I examined whether this was due to the growth in depth occurring under anaërobic conditions. Experiments in this direction shewed that the increase in lysin occurred absolutely independently of whether nitrogen was present or not.

Subsequently numerous authors have been faced with the same fact, and some, Otto and Munter, Nakamura, Doerr and Berger, have taken up the problem for closer investigation. During this it has proved that not alone agar, but all mucilaginosa (gelatine, gummi arabicum, traganth, arrow-root &c.) acted inhibitorily upon the lysis. Here we shall not enlarge upon the details of these investigations, but a brief mention is necessary.

The fact that the substances mentioned have an inhibitory effect upon the lysis without destroying the lysin, all the authors are agreed upon. In what manner this effect takes place none of them dare finally decide. Nakamura who has especially investigated whether all lysins behave in the same manner in the presence of the various mucilaginosa, finds that this is not the case and he employs this in distinguishing the various types of lysin.

Doerr and Berger think it is possible that the inhibitory action of the gelatine is due to the fact that in order to keep it congealed, it has to be kept at a comparatively low temperature. They find, however, that the effect is the same at 37° when the gelatine is fluid. From this they conclude—scarcely justifiably—that it is not the consistency of the gelatine that is the decisive factor. Thus they do not opine that the effect depends upon the individual lysin units being kept away from the bacteria. They support this by means of three arguments:

1) With the same number of bacteria and lysin units, but with a rising gelatine concentration there is an outgrowth of increasing numbers of colonies. Taking the extremes it means that the same number of bacteria and lysin units added to the same volume of gelatine mixture, produces, when the gelatine concentration, for instance, it 1 %, no colonies; when the gelatine concentration is 18 %, numerous colonies, in spite of the fact that in both cases the distance between the lysin units and the bacteria must be identical. Consequently it cannot be the distance that causes the difference. In this argument they overlook the possibility that the lysin may diffuse in a gelatine mixture where the percentage of gelatine is slight, whereas when it is 18 %, this is perhaps not the case.

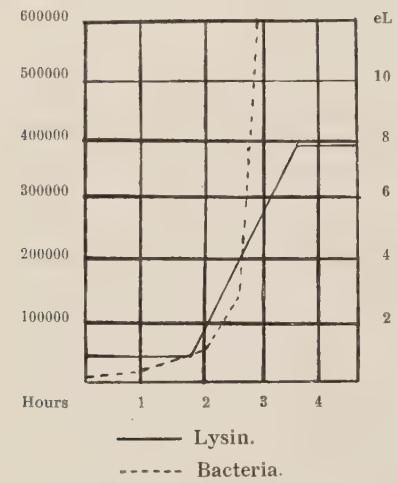
2) The gelatine has the same effect in a melted or fluid state. To this argument it must be contended that gelatine both in a melted and congealed state may inhibit a "movement" no matter whether

this be understood as a diffusion, or is of another description, a chemotaxic phenomenon, for instance.

3) The lysin is certainly not so coarsely dispersed that a purely mechanical point of view can be attributed to these circumstances. Regarding this nothing is known, whatever the order of magnitude of the lysin may be it must be possible to attach a "mechanical" point of view thereto. Consequently, for the time being, we must maintain the possibility that it is just this "mechanical severance from each other" that is the cause of the phenomenon.

After having rejected this possibility the authors mentioned proceed to investigate whether the effect can be attributed to the gelatine adsorbing the lysin. The possibility that an adsorption takes place cannot be excluded, but the intensity of this adsorption is in any case slight. This can be demonstrated by allowing the lysin to stand for some considerable time in gelatine. If we thereupon wash out the gelatine the lysin can be demonstrated almost quantitatively. The effect cannot therefore be due to adsorption. Then they turn to the possibility of the gelatine altering the metabolism of the bacteria. To investigate this they made quantitative experiments in the course of the process of dissolution in the presence of gelatine similar to the experiments already mentioned (see p. 31). Here they discover the interesting circumstance, that no decay of the bacteria sets in, but that the rise in lysin in spite of this proceeds in exactly the same manner as if gelatine were not present. Graphically presented it appears as the figure given. (III).

III.



Here it will be seen that the bacterial concentration does not fall, which as already mentioned denotes that lysin is produced without decay of bacteria. The fact that the bacteria are not dissolved does not, however, indicate that they are living, for if their numbers are determined by a vital count a break in the curve will result. This

break does not occur, in contrast to the break in the earlier experiments with gelatine, until some time after the lysin concentration has become normal.

Otto and Munter repeated these experiments on the course of the process of dissolution in the presence of gelatine and arrives at exactly the same result as Doerr.

Before we proceed to judge what conclusions it is justifiable to draw from these experiments on the course of the lysin process, it is necessary to accentuate a point in the experimental technique that, in high degree, must influence the judgment.

Bacterial concentration, as mentioned, is determined by counting the number of germs that are capable of multiplication, but as this is a product of a process of multiplication and a simultaneous disintegration and as the mutual relations betweeen these two factors are unknown, the result is uncertain. What significance may be attributed to this will be accounted for in the experimental part. Possibly this problem may be solved by counting the bacteria both by means of a vital count and nephelometrically.

What conclusions can then be drawn from these experiments? We observed that the bacterial concentration decreased immediately and rapidly if a maximum initial concentration of lysin was employed. This rapid dissolution of the bacteria did not cause any increase of the lysin concentration. Can this be brought to harmonize with d'Herelles view that the lysin is produced by the decay of the bacteria? Meuli and Doerr are not of the opinion that this is the case. The possibility, however, must be considered that the maximum concentration of lysin may be conditioned by an equilibrium between the formation and destruction of lysin. Before having examined this relation the question cannot be finally decided. The experiments show that the lysin only increases in volume when the bacteria multiply. This agrees with the results of all other authors, and can be classed as a main rule, that without a multiplication of bacteria, no increase of lysin is obtained. The only author who on this point stands alone is Jøtten, who was successful in obtaining an increase of lysin by killing the bacteria.

What is the significance of these experiments in the determination as to whether the lysin is a virus or not?

Meuli personally regards them as decisive in proving that the substance in question is not a virus. His conclusion is as follows:

„Die Lyse hängt sichtlich nicht von der Vorhandensein oder Nichtvorhandensein eines belebten, für die Mikroben infektiösen Mikroorganismen ab, sondern ist die direkte Folge der Erscheinung einer bestimmten Concentration eines noch unbekannten Stoffes in dem Mikroben umspölenden Kulturmedium."

As to the nature to be ascribed to this "substance" he says nothing, thus leaving open the possibility, and to me this seems for the time being the most natural, that we are faced with a new phenomenon, of the nature of which we, up to the present, possess no proof.

In connection with this chapter the difficulties in undertaking experiments of this description will be mentioned.

As already mentioned we have time after time encountered phenomena that coincide so little with what was expected that they may be designated as the antithesis of these. In such a case the technique employed must naturally be most carefully tested and then one is frequently surprised at what has caused the outcome of the experiment to be "topsyturvy".

In my first experiments on the course of the lytic process I made a series of lysin dilutions in bouillon, using increasing lysin concentrations so that each tube contained double as much as the preceeding one. I then seeded all the tubes with a minimum quantity of a susceptible bacterial culture. According to this, it was to be expected that the inhibition of growth would be greatest in the tube containing the largest quantity of lysin. The experiment, however, shewed that in this tube the growth appeared first and was most vigorous. This astounding result was on closer examination proved to be due to the fact that the lysin was not sterile, but contained resistant bacteria. The greater the quantity of lysin added the greater the number of bacteria were obtained in the tubes, and these bacteria, on account of their resistance, were capable of growing in spite of the lysin. Otto and Munter had an absolutely similar experience when they wished to investigate whether the lysins were capable of multiplying exclusively in the presence of killed bacteria. To a suspension of killed bacteria they added lysin, determined the lysin concentration, incubated and again determined the

lysin concentration. This had risen. The result subsequently proved to be due to the fact that the lysin contained lysin resistant lysogenic bacteria. Involuntarily we think of Jøtten's experiment that is the only one resulting in that the lysins can multiply on killed bacteria.

Consequently when working with problems of this description it is necassary to insure against infection not only with heterologous bacteria, but also against infection with homologous.

THE OCCURRENCE OF THE LYSINS.

As will be seen from the following, lysins are very prevalent in nature. Here I do not intend to enumerate all the places where they are demonstrable, a summary of these will be sufficient. In this connection it is of interest first of all to investigate what it is that causes the presence of lysins, and as it has been proved that they are so extraordinarily prevalent, to examine what significance they may be supposed to have in the circumstances under which they occur.

Lysins may to a large extent be demonstrable in the intestinal contents in both healthy and sick animals of almost any description. They may both there, or wherever they are sought, be presumed to be present to a far greater extent than demonstrated. The reason is the following: The lysins, as is known, merely demonstrate their presence by the fact that they dissolve bacteria. If one or the other medium for lysin is to be examined, and it is not beforehand known what species of lysin is concerned, its capacity to dissolve must be investigated against a certain number of various bacteria. In advance, however, it is a matter of course that the greater this number is, the greater is the likelihood of demonstrating lysin in what is examined. As the number of bacteria to be included in an experiment is invariably limited it must be reckoned that lysin may be present without making itself evident against the bacteria employed.

In spite of the fact that the "reaction" for lysin thus is not decisive when negative, lysins are however demonstrated to a very great extent.

d'Herelle has thus demonstrated lysins in healthy persons, horses, cattle of various descriptions, poultry, apes, cats, rabbits and

goats. Wagemans demonstrated lysins in healthy persons, horses, oxen, chickens, guinea pigs, rabbits, in stagnant water, river water and in samples of earth from various quarters. Dumas found lysins in samples of earth, water of the Seine, water from water mains, and in healthy individuals. Other authors, such as Hauduroy, Lisbonne and Carrere, found lysins in similar media.

Upon what circumstances in nature is the presence of lysins then dependent?

d'Herelle is of the opinion that the presence of the lysins in nature depends absolutely upon the simultaneous presence of bacteria, resolving that the lysins by dilution would rapidly disappear if they were not constantly renewed by reaction against living bacteria. He bases this upon the fact that lysins, more especially the more potent, may frequently only be demonstrated where the bacteria they dissolve are or may be presumed to have recently been present.

One must accurately distinguish between the occurrence of the lysin in the animal organism and its occurrence elsewhere in nature. Here the latter will only be mentioned, whereas the significance of the occurrence of the lysin and the conditions governing its presence in the animal organism will later be enlarged upon in detail.

Zdansky who has taken up the question for investigation agrees with d'Herelle that the presence of the lysins in nature depends upon the presence of bacteria. As regards the significance of the lysins these two, however, do not agree.

d'Herelle opines that they play a decisive part in freeing the natural media (the earth and water) from bacteria. Zdansky demonstrates that the conditions governing the reaction of the lysin against bacteria is only to a very slight degree present in nature, excepting in the animal organism. The thermal conditions are as a rule unfavourable and by constant dilution there arises the difficulty that the lysin concentration may become so high that reaction can be induced.

As a source of conveyance for the lysins to nature the contamination of running water from the animal organisms plays an all-important rôle. This Zdansky demonstrates by examining samples of water from the Rhine before and after it has received the contents of a very contaminated canal. He finds that the aforesaid canal contains

large quantities of lysin and simultaneously in percentage, a far greater number of sensitive bacteria in proportion to resistant ones. To what extent the lysin disappears the farther away we get from the sourse of contamination, he unfortunately gives us no information, but the proportion between sensitive and resistant bacteria alters very rapidly, in that the farther from the source of contanination we get, the greater in percentage is the number of resistant bacteria found. Zdansky does not opine that this may be due to the lysins present in the water, but must be attributed to other circumstances. A couple of experiments carried out by him to prove this are not, however, convincing, but his demonstration that the farther we recede from the source of contamination, the greater the number of resistant germs to be found is, in itself, more interesting. All in all, his view that the lysins in nature are subjected to extremely poor reactionary conditions may be said to be exceptionally well-founded.

d'Herelle's opinion that the lysins could actually be used for hygienic purposes in cleansing drinking water of bacteria will scarcely prove correct.

In connection with this section the bacterial species against which lysins have been found will be given.

B. Dysenteriae (Shiga, Flexner, His)
B. Paradysenteriae
B. Coli
B. Typhosus
B. Para typhosus (A and B)
B. Salmonella
B. Typhi murium
B. Proteus
B. Gallinarum (Klein, Moore)
B. Para gallinarium
B. Diphteriae.
Staphylococci
Bacterium of barbone
B. Pestis
B. Flacherie
B. Subtilis

Vibrio cholerae
B. Pyocaneus
B. Friedländer
Enterococci
B. Anthracis
B. Fluorescens
B. liquefaciens
Streptococci.

SEROLOGICAL RELATIONS.

d'Herelle in his earliest communications calls attention to the fact that by intravenuos injections of lysin into experimental animals immune sera can be produced. These immune sera possess properties usual to such, but at the same time, that of being able to counteract the effect of the lysin used in its production. Such a serum is by d'Herelle designated as an antilytic serum.

As mentioned d'Herelle conceives a "bacteriophage" in the following manner; In virtue of its origin it contains:

1) Bacterial bodies in a state of dissolution.
2) Bacterial toxins (endo- and exotoxins).
3) Bacteriophages (arising from the dissolution of bacteria).
4) The lysins of the bacteriophages.

He considers the lytic agent as consisting of a virus (the bacteriophage), that secretes the lysins. These serve to cause the wall of the bacteria to become permeable to virus.

If such a bacteriophage culture be injected intravenously into an experimental animal the following, from a theoretical point of view, should form in its blood:

1) Antibodies against the bacteria: agglutinin, amboceptor.
2) Antibodies against the bacterial toxins: antitoxins.
3) Antibodies against the bacteriophages: agglutinin, amboceptor.
4) Antibodies against the lysins: antilysin.

All authors agree as to No. 1. Identical antibodies are formed as though the actual bacteria had been injected.

In respect to No. 2 it cannot be finally determined whether serum contains antitoxin. The fact is that such antilytic serum will sensi-

tize the experimental animals to toxin injections. If an antilytic dysentery serum be injected into a mouse and this is followed by toxin, it will die more rapidly than the control animal that has not been treated with serum. It has not been decided whether serum contains antitoxin, but in such inconsiderable quantities as to balance the effect of both the dose of toxin administered as well as its own sensitizing effect; this, however, is d'Herelle's opinion. As it is, we may, however, presume that it might also be due to the serum not containing antitoxin.

Regarding No. 3 the fact is that agglutinins against the bacteriophages have not been succesfully demonstrated these latter being invisible and therefore one is prevented from ascertaining any actual agglutination in the ordinary sense of the word. d'Herelle has tested whether the concentration of bacteriophage at the bottom of a tube in which agglutination tests against bacteria have been carried out, has increased. This has proved to be the case, which seems with great weight to conferm that agglutination has taken place.

Opinions differ as to the contents of complement fixing antibody in the serum. Here the difficulty arises that it is necessary to employ the bacteriophage culture as antigen. This contains two inseparable factors the bacteriophages and the dissolved bacteria. If a positive complement binding reaction is obtained we cannot therefore know whether the bacteriophages have reacted against the antibacteriophagic substances, or whether it is the dissolved bacteria that have reacted against the antibacterial substances. d'Herelle clears this obstacle in the following manner: As already mentioned he regards the bacteriophages as a unit, there exists but one bacteriophage, and that capable of assuming virulence against various bacilli. If there exist only one bacteriophage, however. there must also exist but one antibody against it. Consequently it would be a matter of indifference whether in the reaction we use a stapylococcus bacteriophage or a dysentery bacteriophage as antigen. d'Herelle also considers this to be the case. The experiment is as follows: He produces an antilytic serum by means of a dysentery bacteriophage. With this serum he carries out two complement binding tests. In the first, the dysentery bacteriophage is used as antigen. In this experiment he obtains complement fixation, but cannot determine

whether this is due to the dysentery bacteriophage or to the dissolved bacteria. In the other test he uses staphylococci as antigen. In this experiment he also gets a positive reaction, but in this case the complement fixation may be due to the bacteriophage alone, a dysentery serum not yielding complement fixation with staphylococci. The expedient of first absorbing the serum with dysentery bacilli and thereupon testing its complement fixing capacity towards the bacteriophage, he did not attempt.

No. 4 were the antibodies against the lysins secreted by the bacteriophages. d'Herelle regards these lysins as the cause of the bacteriophage being apparently different. It depends upon the secreted lysins whether or not the bacteriophage is virulent against one bacteria or the other. The antibodies formed against the lysins thus are specific, in the sense that they can only neutralize the lysins that have produced them. By this neutralization there arises a fixation between the lysin and the antilysin in the immune serum. The bacteriophages themselves are, per contra, uneffected by the fact that their lysins are fixed. d'Herelle proves this by neutralizing a lysin quantitatively and thereupon adding it to a bacterial culture. To begin with the culture will grow as were there no bacteriophage present, but when the bacteriophage has had sufficient time to secrete new lysins, it will again be able to attack the bacteria and the culture will be dissolved.

All what has here been stated of the serological relations of the bacteriophages has been advanced by d'Herelle and based upon fine experiments, experiments that for those who have worked on similar matters, impress one as having been more successful than is usual with such experiments in general. It must be emphasized, to d'Herelles great merit, that his theory is based upon experiments that in such a high degree has been reproducible, as has proved to be the case. It does not detract from his merit that all details have not been verified by other authors. To what an extent other authors have been able to confirm d'Herelle's result will be the subject of the following investigation.

Regarding the fact that immune sera, produced by injection of bacteriophages contain antibodies against the bacteria from which the bacteriophage culture is a solution, all authors agree. I, per-

sonally, have occupied myself but little with these serological questions, but this point I can fully endorse.

Antibodies against bacteriophages and against the lysins secreted by the bacteriophages are treated by the majority of authors indiscriminately. The reason for this is, that the majority do not agree with d'Herelle that the phenomenon is due to a virus.

That the antilytic sera are capable of counteracting the bacteria-dissolving capacity of the lysins*) all agree. Likewise all agree, that this effect is specific, i. e. that an antilysin only neutralizes the lysin used in its production or lysins that may be regarded as identical to it.

If a mixture of various lysins is injected, a mixed serum is obtained capable of neutralizing separately the lysins used. This has been made the subject of special investigations by Wagemans and Watanabe in connection with their endeavours to separate the various lysins. There are, however, exceptions. Thus Wagemanns calls attention to the fact that some lysins are only neutralized with difficulty. Otto and Munter find that antilytic sera, produced by means of very potent lysins, may be unspecific in the sense thatt hey in part can neutralize other lysins. Simultaneously they certainly find that very potent lysins may also attack heterologuos bacteria.

How then are we to conceive the fixation between the lysin and the antilysin? Can it be compared with the fixation toxin-antitoxin or to trypsin-anti-trypsin? Is the action of the fixation such that the lysin is destroyed?

That the latter is not the case was, as mentioned, demonstrated by d'Herelle. Costa Cruz has demonstrated that by filtration through Berkefeld filters the lysin can be separated from the anti-lysin.

Wagemans contends that the fixation lysin-antilysin does not take place in the same manner as the fixation toxin-antitoxin, lying stress upon the fact that it takes a much longer time. Otto and Munter are, however, of the opinion that it closely resembles the toxin-antitoxin fixation because it is reversible and because the fixation

*) The designation lysin is again used as a term for the whole phenomenon whether or not this be due to a virus or something else.

takes place more rapidly at 37° than at a lower temperature and becomes closer after a time.

Watanabe and Prausnitz do not believe that it can be a question of the neutralization of a ferment. If we neutralize a lysin partially it can be demonstrated that the lysin units remaining are uninjured and this would not be the case if it were a ferment, all parts of which would have been equally neutralized.

According to Wagemans and Watanabe the presence of complement is a matter of indifference in the occurrence of the fixation.

Watanabe and Prausnitz have demonstrated that with bacteria one can only absorb the anti-bacterial antibodies in an antilytic serum, but not the antilytic. Thus Prausnitz concludes that the lysins cannot be "splitter" of the bacteria, (Bail). If they were, one ought to be able, with undamaged bacteria to absorb both the antibodies due to the lysins as also those due to the bacteria.

Before ending this chapter with a mention of the views of the various authors regarding the complement fixing antibodies I should like to speak of the so-called "serum-fast" lysins.

By a serum-fast lysin we understand a lysin that is not neutralized by an antilytic serum produced by the injection of the same lysin. Wagemans and Prausnitz have produced such serum-fast lysins by treating the lysin with ever increasing quantities of antilytic serum and have found that it was enabled to withstand constantly increasing quantities thereof until finally it could not be neutralized at all. Seisser, Otto and Munter have undertaken identical experiments but without success. They state that the reason why we get an apparently serum-fast lysin is that every lysin by passages over bacteria will increase in potency and that it cannot be decided whether it is this that occurs or whether it is the lysin that becomes serum-fast.

By many authors the fact that serum-fast lysins can be produced is used as an argument that the lysins are vira, contending as they do, that only a living organism can be possessed of such adaptability.

As regards the contents of the specific complement-fixing antibodies peculiar to the lysins in the antilytic sera, nobody has been successful in verifying or invalidating d'Herelle's results.

Wolmann and Goldberg have attempted to utilize the possi-

bility first of absorbing the antilytic sera with bacteria and thereupon to institute complement binding reaction with the absorbed serum and lysin. Their experiment is, however, of such a description that from it it is not possible to draw any conclusions. They entirely lack control experiments as to the self-inhibition of the serum, and in their communication there are printer's errors, the confusing influence of which cannot be elucidated.

Gratia and Jaumain have endeavoured to demonstrate that the complement fixing antibody, common to all lysins, is not due to the lysins but to another agent contained in the culture. They point out that the lysins, besides the actual lytic agent, contain a substance as complicated as bouillon and the substances that the bacteria during their growth have produced therein. In two of five experiments they arrive at the result that serum only reacts with the homologous lysin. In 3 out of 5 experiments they come to the conclusion that serum, besides with the homologous lysin, yields complement fixation with heteorologous lysins, consequently the same result as d'Herelle, but in these three cases they also got positive reaction if they substituted old bacterial cultures for the lysins, thus without the presence of lysin. Consequently the complement-fixing antibody in the antilytic sera is not specific to the lysins.

Brynoghe and Maisin adhere to d'Herelle's view that the antibody is common to all lysins.

Otto and Munter examine the reaction of various antigens, some with an antilytic serum, some with an antibacterial serum. The differences they find are, however, so slight that the conclusions to be drawn cannot be presumed to be tenable.

The question as to whether the antilytic sera contain a complement-fixing antibody specific for the lysins must therefore be as yet considered as undecided.

LYSIN THERAPY.

Can the lysins be used therapeutically?

This question, that for d'Herelle as for the majority of other authors has been of principal importance, is still the subject of lively discussion.

If we wish to examine the possibility of employing lysins in therapy, it is natural primarily to observe what rôle they may be supposed to play during ordinarily occurring diseases and epidemics, and thereupon to note their effect on artificially produced infections in animals. The animal experiments, as d'Herelle justifiably has observed, are of secondary significance because one has always to make allowance for the susceptibility and resistance in the experimental animals differing from that present in natural infections.

What part is consequently played by the lysins during naturally occurring diseases and epidemics?

d'Herelle has made this question the subject of intensive studies. I do not intend to enlarge upon these, being, as they are, reported in detail in his monograph, but a contemplation of the main results will be of interest.

The main principle of d'Herelle's researches is as follows:

If one invariably, when an epidemic — or a disease in one single person — is about to die out, can demonstrate lysins and these can never be demonstrated while the disease rages, it is propable that there is a causative relation between the presence of the lysin and the cessation of the disease. This probability is realized if, by introducing lysin, one can cause the epidemic or disease to cease.

d'Herelle's material is copious and heterogeneous an his observations almost always coincide with what he expected to discover. In virtue of this they are very convincing and one may wonder that they have not, to a far greater extent than is the case, been tested by other authors.

The diseases that d'Herelle has investigated are the following: Typhoid, para-typhoid, dysentery, avian typhosis, haemorrhagic septicaemia in buffalos and oxen (barbone), bubonic plague and flacherie.

Typhoid, paratyphoid and dysentery he investigates by daily examining the feces of the patients for lysin against the microbe causing the disease and also for some others, measuring simultaneously the potency of the lysins. Moreover, he examines specimens of the feces to discover pathogenic bacteria. Thereupon he compares the lysin curve thus found with the temperature curve in patients suffering from typoid and paratyphoid and with a curve shewing the number

of stools in dysentery patients, and between these he invariably discovers a definite ratio.

He classifies the cases into 4 groups.

1) Severe cases, in which the patient's condition, however improves rapidly. In these cases he found at the commencement of the disease no lysin, but from the moment the state of the patient began to improve he discovered a lysin that acted rapidly both against bacteria cultivated from the actual patient and against laboratory strains. The lysin remained for a time during convalescence and simultaneously the bacteria disappeared from the stools.

2) Cases, where the disease is protracted. Here lysin was found that only acted weakly against bacteria cultivated from the patient. As improvement sets in the lysin increased in potency.

3) Protracted cases and cases with relapses. The potency of the lysin might here be considerable against laboratory strains, but was always weak against bacteria cultivated from the actual patient, though its potency underwent great fluctuations. These fluctuations corresponded, however, invariably to the condition of the patient, inasmuch as when the patient improved the lysins increased in potency and vice versa.

4) Very mild cases, i. e. only some few attacks of diarrhoea. Here a potently active lysin was always found.

In avian thyphosis d'Herelle discovered quite identical circumstances. Avian typhosis is an epidemic disease that, as a rule has a fatal issue in the course of a couple of days. Should the fowls in a poultryyard be attacked, almost all die. If the excrements are examined lysins are scarcely ever found that are effective against the bacteria causing the disease. Should it happen that a single fowl recovers, it is almost invariably discovered that a potent lysin is present in its excrements, and simultaneously the epidemic will die out, the remaining fowls becoming infected with the lysin and the disease is thereby brought to an end, also in regard to them. If a lysin is introduced artifically into an infected stock of poultry the same result is obtained.

Exactly the same thing happens with haemorrhagic septicaemia in buffalos. The disease has a mortality of 75—95 %. Should one single animal recover the remainder will as a rule also be saved as

the lysin that is then found in that animal will be transferred to them in some way or other.

The research on bubonic plague specially concerns rats. d'Herelle here discovers the same accurate conformity between the presence of lysin an the cessation of the disease.

As the result of these researches, that in their detail are beautifully elaborated, d'Herelle advances the following theory:

The bacteriophages are always present both in healthy as well as sick animals. They multiply on the bacteria always present in the intestine of the animal. The virulence for these fluctuates and it is always capable of becoming virulent for new bacteria that might be introduced. On this capacity of assuming virulence for the various bacteria and the velocity with which this occurs, actually depends the course of each infection. Should this occur rapidly and should the virulence be high perhaps there will not even be an outbreak. If this takes place slowly and incompletely, a more or less violent case is recorded. Should the capacity of the bacteriophage to assume virulence for the bacteria in question fail, a case with fatal issue results.

Immunity whether this is beforehand present or acquired during the infection, thus depends upon the presence of bacteriophages.

What is the duration af such and immunity? d'Herelle's answer to this is that it lasts as long as fresh infective matter is introduced. An example will illustrate this: A chicken that harbours a highly virulent bacteriopbage lives in a poultry farm infected with avian typhosis. To the chicken thus, typhoid bacilli are constantly introduced its bacteriophages dissolve these and thereby retain their virulence. If the chicken is placed in a spot where there are no typhoid bacilli the bacteriophage loses its virulence for typhoid bacilli from want of »nourishment«, and continues to vegetate on coli or other bacilli present in the intestine of the chicken.

d'Herelle thus puts forward what he himself terms a heterologous immunity as opposed to the ordinary conception of immunity, disignated by him as homologous. A better designation for the immunity produced by the bacteriphage, is employed by d'Herelle in his earlier communications, calling it as he does, enterogenic immunity as opposed to the haemogenic.

The fact that an injection of bacteriophages may produce immunity, that does not depend upon the presence of bacteriophages, will later be mentioned.

The haemogenic immunity is only partially recognised by d'Herelle who contends that one has never seen immune sera dissolve or kill bacteria, and that in the measurements of the antibodies contained in the immune sera we do not possess any gauge of their antibacterial action.

How can a bacteriophage that is essentially only found in the intestinal canal, influence the bacteria found in the blood? This occurs in the following manner: The bacteriophage is an ultravisible virus that secretes a lysin. This lysin is resorbed to the blood causing here an increased phagocytosis.

d'Herelle does not deny the presence of bacteriophages in the blood, but from his writings, it is not clearly shewn what significance he here attributes to them. Personally he has demonstrated them in the blood of mice suffering from typhous septicaemia.

This is the rôle that d'Herelle ascribes to the lysins in the question of immunity. As already mentioned there exist but a few other communications on the relation of the lysins to the course of infectious diseases. A number of authors have demonstrated lysins in patients with various diseases, but only a few have undertaken investigations comparing them with the course of the disease.

Hauduroy found on examining 9 typhoid patients that lysin can only be demonstrated when the patients are about to recover, never when the temperature is high. He gives, however, no information as to when and how frequently he examines specimens of feces. This same author examines the blood of typhoid convalescents for lysin. He finds lysin both in blood tests in which there are bacteria as also in those where none are present. The lysin in the former dissolves the homologous bacilli only, and the lysin in the latter dissolves both the homologous and the heterologous strains. He himself remarks on the paradoxicalness of finding lysins but no bacteria in one and the same blood specimen, but explains it as follows:

By closer examination of the blood cultures that yield negative cultivation results, one find by daily microscopic examinations typhoid bacilli that increase in numbers until the 6th day, whereupon they

gradually disappear. These bacteria it was not possible successfully to cultivate further from the original culture. He therefore opines that in the same specimen of blood, one has taken both bacteria and lysin, and that these react against each other in the culture. From this he concludes» »qu'a la fin d'une fièvre typhoide, au moment ou les hémocultures sont tantôt positives tantôt negatives la présence du microbe dans le sang est regleé par la présence du bactériophage et par son pouvoir lytique«. He never finds lysin in the blood of healthy persons and animals. In contradiction to this conclusion, stands the fact that the majority of other authors agree that bacteria and lysin do not react against each other in the blood.

Bachmann found lysins in typhoid convalescents. These lysins reacted always weakly against typhoid bacilli, he therefore is of the opinion that they cannot be employed for the treatment of typhoid patients. His finding likewise can scarcely be cited as an authority in favour of d'Herelle's theory.

Debré and Hagenau found lysin in 3 out of 6 dysentery cases, in 3 out of 10 cases of typhoid, an also in numerous patients with diseases not emanating from the intestinal canal. The lysins that originated from typhoid patients never dissolved typhoid bacilli.

Hajos examined dysentery and typhoid patients after these diseases. In patients he never found lysins that dissolved the homologous strain which, however, was the case with convalescents and healthy persons. Furthermore he found that lysins taken from various intestinal sections differ.

Ciuca examined 5 cases of cholera throughout the whole illness and convalescence. He found that the feces both during the illness as also during the convalescence contained lysins that dissolved coli and dysentery bacilli, but never lysins that dissolved cholera vibrios in spite of the fact that he used 17 different strains in the demonstration. Thus he concludes that the lysins are something unspecific, to the influence of which dysentery and coli bacilli are especially susceptible.

Cuica and Petrovani examined 4 cases of infantile gastro-enteritis, 5 cases of cholera and 4 cases of bacillary dysentery. The stools were examined for lysin, sensitive and resistant bacteria. The lysin was found in all the 13 cases. In the dysentery and gastro-enter-

itis patients lysin was found that dissolved dysentery and coli bacilli. In all the cholera patients lysins was found, but it could never dissolve cholera vibrios. Resistant an lysogenic bacteria they were not successful in demonstating. If a dissolution af the bacteria took place in the intestine by means of lysin one ought also to find resistant bacteria, but this was not the case. Consequentliy the authors concluded that the dissolution was due to totally different causes (digestive diastases, decomposition products, etc.). In the intestine only the final result of the dissolution is found namely the lysin. This also explains why the lysin so rapidly disappears when the patient recovers. The bacilli disappear first and thereupon the products formed by their dissolution, viz. the lysin. Hereby they arrive at the same result as Doerr, and that is, that the lysin itself with the formation of the resistant bacteria is a laboratory phenomenon concerning the natural occurrence of which nothing is known. Doerr's researches in this field have already been mentioned, likewise Zdansky's.

Willazon found plague lysin in plague infected rats.

Kuttner found in two typhoid carriers lysins that dissolve the homologous bacteria. In one typhoid convalescent she demonstrated a lysin that reacted strongly against the homologous strain. In spite of this the patient became a bacillary carrier. These cases show that the presence of even a potently active lysin does not condition the organism becoming free from bacteria.

Callow found lysin in 6 cases of 14 staphylococcus infections. In two cases the lysin found dissolved the homologous strains, in remainder the heterologous strains only.

Fabrii found in a urine of a prostatic patient a lysin that dissolved coli bacilli. In the urine staphylococci bacilli only were found (originating from prostate). The lysin was incapable of dissolving staphylococci.

Petrovani demonstrated lysin in pus from the the crypts of the tonsils of patients with scarlatina and other cases of angina. The lysins found were tested against coli bacilli only, not against the bacteria found in the pharynx.

Pons discovered lysin against pyocyaneus in pus from otitis cases.

Watanabe found staphylococcus lysin in the pharyngeal secretion of diphtheria patients.

Hauduroy's results speak in favour of the correctness of d'Herelle's theory. As already said, they require a thorough testing and even if they should prove to be correct they are not unequivocal.

Hajos' results point in a similar direction, but there is a lack of statements as to how many specimens of feces from each patient were examined.

Bachmanns, Cuica's and Cuica and Petrovani's and more especially Kuttner's researches denote that there is no causative relation between the presence of lysin and the cessation of the disease, respectively the disapperance of the bacteria.

My own researches in this field will be accounted for in a subsequent chapter.

They go to shew that circumstances are not always as found by d'Herelle, for a potent lysin against the homologous bacteria may be present from the commencement of the disease, even if the course is protracted and serious.

ANIMAL EXPERIMENTS.

Through these it was endeavoured to elucidate whether the lysins by artifically produced infections had any therapeutic effect. As their practicability in this respect to a great extent, depends upon their resorption and excretion it is natural primarily to look at these.

Appelmanns is of the opinion that the lysin when administered per os cannot be resorbed. If we inject the lysin subcutaneously it is resorbed and may be demonstrated in the blood and the majority of the organs. It is rapidly execreted through the feces and the urine.

Kabeshima injects lysin and demonstrates it in the gall in from 30 minutes to 16 hours, seldom 24 to 48 hours after injection.

Otto and Munter demonstrate lysin in the blood 7 hours after an intra-peritoneal injection of lysin.

Bordet and Ciuca demonstrate lysin in the blood 24 to 48 hours after injection.

Wertemann found lysin in the blood of rabbits and guinea pigs

until the 5th day and in frogs until the 7th day after intravenous injection of lysin. He determined the lysin concentration in the blood of these animals and found that it decreases steadily.

Doerr and Grüninger demonstrated that a very short time after intravenous injections lysin is found in the gall and urine. The quantity found depends upon that injected. With the dose usually given only very minute quantities are found. They condemn the dosing in cubic centimeters, if the lysin concentration is not simultaneously stated. After 14 hours they could no longer demonstrate lysin in the gall. They were unsuccessful in making rabbits and guinea pigs bacteriophage carriers.

Subsequently they endeavour to inject simultaneously lysin and coli bacilli intravenously. If coli bacilli are injected alone, one seldom produces bacilli carriers. If on the contrary, they injected lysin and bacteria simultaneously they produced far more frequently cholecystitis with the resultant lasting secretion of bacilli. This remarkable finding is possibly explained by the fact that the bacilli do not react against the lysins in the blood. In the gall, per contra, there occurs a reaction that on account of the peculiar properties of the former takes the following course: That the bacteria are not dissolved, but transformed into resistant bacteria. This may, to an especial degree, be presumed to be the case with human gall that is inspissated and mucous and therefore acts as a mucilaginosum. For this reason it would be unwarrantable to inject typhoid patients with lysin, because thereby theoretically viewed one would merely arrive at turning the majority into bacilli carriers. Moreover for human beings larger doses would be required and this would be impracticable owing to the toxicity of the lysins.

d'Herelle and Eliava shewed that by inoculating themselves, oxen and fowls with dysentery and plague lysins, but a few hours after the injection, the lysins could be demonstrated in the feces.

According to these authors the resorption an secretory conditions are thus relatively good for a therapeutic application of the lysin, it being quickly resorbed, equally distributed in the organism and secreted relatively slowly.

Almost all authors agree that the lysins when administrered per os pass uninjured into the intestine.

Regarding the toxicity of the lysins a number of researches have been undertaken that have not, however, conduced to agreement, some considering that it is considerable, and others that it is practically speaking nil. Auto-experiments by d'Herelle and Eliavia, Otto and Munter and Gjørup shew that the latter at any rate may be the case. d'Herelle has demonstrated that freshly prepared lysins are fairly toxic, whereas lysins that have been stored for 1 or 2 months do not produce reaction of any description, whether administered per os or injected subcutaneously.

What is the effect of the lysins on artificially brought about and naturally occurring infections?

Here there is a question of examining their effect when administered prophylactically, and when given after the disease has broken out.

Does the lysin produce immunity and if so how does this occur, how does it act and how long is its duration?

Of researches concerning these points d'Herelle's ought to take precedence.

We have already seen that during epidemics (avian typhosis and septicaemia in cattle) an immunity might occur that was due to the presence of lysins in the digestive canal of the animals. This immunity lasted whilst to the animal fresh infectious matter was conveyed.

d'Herelle then investigated the result of injecting lysin into animals, that for the moment are not exposed to infection, but subsequently are. These immunity experiments immediately concern buffalos. Haemorrhagic septicaemia in these is, as is known, a disease with a mortality of almost 100 %. 0.0002 c. c. of a culture of infectious matter will with certainty kill an animal weighing 100 kilograms in the course of 36 hours.

During these experiments to determine what dose of lysin produced certain immunity d'Herelle discovered that no matter what dose was administered, the injection caused the animal to become sensitized to infection with the infectious matter. This sensitiveness later fairly suddenly broke into a complete and lasting immunity. An

incubation period was also discovered, the duration of which depended upon the quantity of lysin injected. The greater this was, the longer the incubation lasted. Its duration after an injection into a buffalo of 5 c. c. was from 15 to 18 days.

Now how can these circumstances be explained?

As already mentioned the immunity that sets in when infectious matter is constantly conveyed to the animal depends upon the fact that the intestine harbours a bacteriophage that is constantly maintained by the bacteria introduced and at the same time destroys them. When bacteria are no longer conveyed to the animal the lysin disappears. The lysin also disappears very rapidly when injected into animals not exposed to infection. It cannot be the presence of the lysin that conditions the immunity. d'Herelle also demonstrated this by destroying the lysins, either by subjecting them to glycerin or heat. If a lysin thus treated, be injected the same result is obtained as with ihe untreated lysin.

Consequently it is not the living bacteriophages that condition the immunity. It might then be presumed this was due to one or several of the following factors. The killed bacteriophages, the lysins secreted by the bacteriophages or the dissolved bacteria. d'Herelle considers that it cannot be these latter, because he does not find antibacterial antibodies in the blood of the immunized animals (agglutinin, amboceptor, opsonin). The immunity may possibly be due to a special bearing between the lysins secreted by the bacteriophages and the bacteria dissolved by them. That the mechanism differs from that taking effect when animals living in infected surroundings are immunized, he considers as granted.

The very same immunizing circumstances as in the case with barbone, are demonstrated by d'Herelle in avian typhoses. He likewise immunizes rabbits against dysentery.

Kabeshima was successful in immunizing rabbits against a dose of dysentery bacilli many times fatal. He does not admit to finding any incubation period.

Bordet and Ciuca have by means of intraperitoneal lysin injections into guinea pigs immunized these against a dose of coli fatal in 8 hours.

Hauduroy demonstrates that rabbits can be sensitized by injec-

tion of staphylococcus lysin so that they become more susceptible to chance skin infections and die after injection of staphylococcus cultures in doses that fail to kill control animals of the same weight.

Metalnikow's experiment with galleria melonella deserves special mention. Galleria melonella (the larva of the wax-moth) is very susceptible to injection with bacteria of the typhoid-coli group. One of these as a rule causes death in the course of 12 to 15 hours. The reason for this is that the usual defence of the larva against infection, a potent phagocytosis, fails entirely when opposed to these bacteria. After injection of lysins against bacteria of the colityphoid group the phagocytosis sets in also against these, to such an extent as has never before been observed. The experiments are interesting, not alone because they shew that the larva can be actively immunized, but more because they go to shew that the effect, in this case, is due to the fact that they produce an increased phagocytosis.

Willazon obtains a partial immunity against plague in rats by injection of lysins.

d'Herelle's experiment has thus been confirmed by all these authors and there are no communications tending in an opposite direction. Thus it appears conclusive that animals can be immunized by lysin injections.

The question as to how this immunity sets in is not yet determined. The possibility that it is simply a vaccination effect, due to the dissolved bacteria, is just as likely as the possibility that it is attributable to bacteriophages or the lysins secreted by them. The solving of the problem lies perhaps between the two, as bacteria dissolved by bacteriophages perhaps, thereby gain an increased vaccination effect.

As regards the duration of the immunization produced by lysin injections we have but one single communication. d'Herelle together with Louet has found that in buffalos it is still sufficient after the lapse of a year.

d'Herelle has demonstrated that the transfusion of 500 c. c. of blood from an immune buffalo to another can immunize it.

Before passing to a review of the results of lysin therapy some few remarks regarding it might be adequate.

Can the lysin produce harmful effects without counteracting the infection?

This question is, as already mentioned, answered in the affirmative by Doerr and Grüninger shewing as they do that, by simultaneous injections of lysins and bacteria, cholecystitis far more frequently sets in than by injection of bacteria alone. The cholecystitis that sets in is produced by the resistant bacteria that, besides being unsusceptible to the lysin, are more toxic than the original (Bordet and others).

In opposition to this Marcuse contends that the resistant bacteria do not convey any danger, their resistance only making itself evident against the lysin, but that in their relation to the organism they do not differ from ordinary bacteria. Their resistance is even specific, to such a degree, that it only becomes evident against the lysin producing it, whereas it may easily be dissolved by other lysins. Whether the fact that the resistant bacteria are inagglutinable denotes that they also have acquired a certain resistance against the defences of the animal organism, is an open question. The fact that they can produce the usual antibodies as agglutinin and amboceptor seems to speak against this.

Even if it should prove that the resistant bacteria behave as do other bacteria against the defences of the organism, Doerr and Grüninger's discovery of the more frequent occurrence of cholecystitis remains and warns us to be cautious.

Which diseases are most suitable for lysin treatment?

As will be seen in the subsequently reported cases, the favourable results are, in almost all cases, among the localized diseases, such as cystitis and staphylococcus affections in the skin. It would appear that here good results are far more frequent than in the more universal intestinal affections. This is perhaps due to the lysins being brought into more direct contact with all bacteria, and that their concentration is not immediately reduced by the supply of fresh secretions and the removal of the old. In therapy, Marcuse contends, we must endeavour to approach as closely as posssible to the test-tube experiment. That is to say, one must endeavour to maintain the lysin concentration as high as possible, bring the lysin into as close a contact as possible with all the bacteria and see that the

reaction is kept alkaline or neutral. When the lysins, in spite of these conditions not being complied with, have yielded favourable results it is perhaps not so much due to the lysins as to tha vaccination effect of the dissolved bacteria.

Marcuse here touches on a question of great interest. The opposite question might just as well be posed, how much of the usually observed vaccination effect is due to the prensence of lysins. There can scarcely be any doubt that many of the usually used vaccines contain lysins that fully tolerate the procedures that the vaccine generally undergoes. The fact that the vaccination treatment is very capricious, sometimes of excellent effect at others times absolutely without effect, might easily be explained by stating that some vaccines contained an active lysin while others did not.

One difficulty in lysin treatment is that it is not always equally easy to procure lysins that dissolve the bacteria one wishes to destroy. The greater a collection of lysins one has, the greater is the likelihood of finding a lysin capable of dissolving the bacteria in question. This however at times fails. This may be remedied by as far as possible making ones lysins polyvalent. Another expedient is to make use of Borchardt's method in the production of lysins or, as Doerr has proposed, to feed chickens with the bacteria against which a lysin is required. According to his researches one will frequently find the lysin required in the excrements of the chickens.

How then does the lysin act when it is used therapeutically, in a restricted sense, i. e. after a disease has broken out?

On this subject there are numerous communications. Some authors have recorded excellent effects, some none at all.

d'Herelle treated 7 cases of bacillary dysentery in children. Some of these were very severe, others mild. In all cases the examination of feces before treatment shewed that there were no lysins present against the phatogenic bacteria. The patients were all treated with a single dose of lysin of 2 c. c. subcutaneously. In all cases the effect was identical. In the course of from some few hours to some few days the diarrhoea ceased and the patients recovered. It is not stated at what stage of the disease the treatment was instituted. In respect to one of the patients it was stated that just before a sister had died of dysentery.

Simultaneously it must be reported that in all cases of avian typhosis (100 cases) in which the treatment was instituted, d'Herelle was successful in arresting the infection in the individuals attacked.

Beckerich and Hauduroy treated 7 cases of typhoid and paratyphoid both severe and mild. In one of the severe cases, the patient died in spite of the treatment. In the remainder a lasting fall of temperature was recorded in the course of 48 hours. Simultaneously they were given up to 5 c. c. lysin per os and up to 2 c. c. subcutaneously. The treatment was instituted at the following junctures: 18, 9, 20, 9, 23, 10 and 14 days.

Brynoghe and Maisin treated 6 cases of furunculosis and anthrax with injections of from 0.5 to 2 c. c. The effect becomes evident in 48 hours and consists of a rapid resorption of the tumour and healing without scar. In the farther advanced cases the pus was emptied. A slight reaction took place at the spot where injected and in the severe cases slight fever, (resorption).

In a subsequent work the authors, however, report that as a rule a reaction sets in consisting of a brief rise in temperature to between 39^0—40^0, slight headache and insomnia, swelling, flushing and irritation of the skin at the spot of injection. The reaction runs proportionally with the size of the dose.

Gratia and Jaumain came to quite similar results in the treatment of anthrax and furunculosis. They described besides the ordinary reaction and a reaction at the spot of injection similar to the aforementioned, a reaction at the spot attacked consisting of a bright red margin around the focus and the old scars.

In one case reaction also set in in an old appendicitis scar from which two sutures were ejected. They compare the reactions with those of tuberculin. Whether it is due to the lysin or the dissolved bacteria they dare not determine.

Davison treated 12 cases of bacillary dysentery in children with lysin. All cases were treated late and the author calls attention to the fact that $^2/_3$ of dysentery deaths in children take place within the 12^{th} day. Of these 12 children only 4 came under treatment before the 12^{th} day, the remainder thus should have had the possibility of recovering without treatment.

Among these 12 cases 9 were severe. Of these two lived the re-

mainder died. All three mild cases lived. In one case only the treatment had no apparent effect.

The doses employed were very large, from 5 to 198 c. c. per single dose administered per os or per rectum. A three months old child received in this manner 1381 c. c. in the course of 10 days. The slight effect cannot therefore be attributed to small doses.

Neither was it because the bacteria were resistant against the lysin used, for bacteria cultivated from the patient could be dissolved by it. It might be imagined that the bacteria during the treatment had become resistant, but the contrary was the case, the bacteria cultivated from the patient, just after the cessation of the treatment, were more easily dissolved than bacteria cultivated from the patient, prior to the beginning of the treatment.

In spite of this Davison, however considers that on account of d'Herelle's good results it is not possible finally to establish the want of effect in the lysin, but he awaits developments.

Otto and Munter do not obtain any positive result in the treatment of dysentery. The lysin was administered per os, as a clyster and intramuscularly.

Guorgerot and Peyre treated 5 cases of pustulous sycosis and 2 cases of furunculosis with lysin. The treatment was undertaken locally, the pustules being perforated with a pipette-ful of lysin. All pustules were carefully treated, a local reaction set in round the pustule that rapidly dried up and disappeared.

Jøtten is of the opinion that the treatment is harmless. In the treatment of some typhoid patients he did not obtain any positive effect.

PERSONAL RESEARCHES.

INTRODUCTORY EXPERIMENTS.

The introductory experiments on d'Herelle's phenomenon were carried out with a coli strain (CB) and a corresponding lysin that were obligingly given to the Serum Institute by Bordet.

This lysin that when I received it in September 1920 yielded plaques extending throughout the whole thickness of the culture leaving the agar plate immediately bare retained this characteristic until March 1922. A change then set in and the plaques became so tiny that they were scarcely visible and at the same time they no longer penetrated throughout the whole thickness of the culture but occupied only the most superficial part of this. The reason that the plaques thus changed character might at the outset be presumed to lie either in the lysin, in the bacteria or in a change in the circumstances under which the cultivation was undertaken.

Attempts to produce plaques of the previous appearance by changing the experimental conditions (p_H, percentage of peptone, tp, consistency of the agar or degree of moisture) to produce plaques of the original appearance failed.

It may be regarded as most probable that the altered appearance of the plaques was due to changes in the bacteria, for the culture unaffected by the lysin, simultaneously with the change in the appearance of the plaques, became somewhat drier and rough on the surface.

The lysin received from Bordet was tested immediately on receipt against one typhoid, two paratyphoid, one dysentery, one paradysentery and one coli strains without it being possible to dissolve one of them.

Thereupon I commenced examining the feces of some patients for lysin. The technique was that stated by d'Herelle: A lump of feces the size of a pea is suspended in bouillon (10-20 c.c.), placed in the thermostat for 24 hours and filtered through a Berkefeld filter.

From the filtrate 1-2 c.c. were transferred to a bouillon culture of the bacteria to be dissolved and from this mixture is seeded on an agar plate with a goosenecked glass rod.

(This technique had later to be modified. Here mention will only be made of the results yielded by the modified technique).

			Bacillus	Lysin	Disease
1.	J. N.	Excr. I	Typhoid.	0	Typhoid.
		» II	Coli A.	anti Coli A	
2.	C. B. J.	» I	Typhoid.	0	Typhoid.
		» II	Coli B.	anti Typhus	
3.	M. W. J.	» I	Typhoid.	0	Typhoid.
		» II	Coli B.	0	
4.	Joh. N.	» I	?	0	Typhoid.
		» II	Coli.	0	
5.	J. S.	» I	Coli B.	0	Typhoid.
6.	B.	» I	Coli A & B.	0	Fermentation
		» II	Coli B.	0	dyspepsia
7.	B. P.	» I	Coli.	0	Acute diarrhoea
		» II	Gram + cocci.	0	
8.	S.	» I	B. faecalis alk.	anti Coli B.	Acute diarrhoea.
		» II	Coli B.	0	
9.	N. M.	» I	Coli B.	anti Coli B.	Acute diarrhoea.
		» II	B. faecalis alk.	0	
10.	C. N.	» I	Coli B.	0	Acute diarrhoea.
		» II	Coli B.	anti Coli B.	
11.	M. M.	» I	Coli B.	0	Acute diarrhoea.
		» II	Coli B.	anti Coli B.	
12.	C. O.	» I	Coli B.	0	Colitis ulc.
13.	A. H.	» I	Coli A.	0	Typhoid.

The bacteria against which I tested the filtrates were, in the first place, bacteria cultivated from the patient's own feces, but in addition the effect of the lysins found was also examined against the bacteria found in all the patients. The results are given in the table above. As will be observed stools from 13 patients were examined. From the majority of patients two stools were examined (I and II) from three patients only one stool. In the second column the bacteria found in the stools is given; in the third column it is stated

whether lysins was found and if so against what bacteria. The disease of the patient is given on the extreme right.

In 6 cases lysin was found, in 4 against coli B., in one against coli A., and in one against typhoid.

The 6 lysins found were thereupon tested against all the bacteria discovered and in addition against some few others.

Lysin from patient No. 1 proved to be effective against:

Coli A cultivated from patient No. 1
Typhoid » » » » 1
» » » » » 3
Coli B » » » » 11
Parathyphoid » » » bacilli carrier K. M.

Lysin from patient No. 2 proved to be effective against:

Typhoid cultivated from patient No. 1
» » » » » 2
» » » » » 3
Coli B » » » » » 11
» » » » » » » 12
» » » » » » » 5
Paratyphoid » » » » bacillus carrier K. M.

Lysin from patient No. 8 proved to be effective against:

Coli B cultivated from patient No. 8

Lysin from patient No. 9 proved to be effective against:

Coli B cultivated from patient No. 9

Lysin from patient No. 10 proved to be effective against:

Coli B cultivated from patient No. 10
» » » » » » 11

Lysin from patient No. 11 proved to be effective against:

Coli B cultivated from patient No. 11

Thus it is shewn that the lysins were not specific against the individual bacteria, for lysin from patient No. 1 dissolved Coli A, Coli B, typhoid from 2 different patients and paratyphoid B. Lysin from patient No. 2 dissolved typhoid from 2 different patients, Coli B from 3 different patients and parathyphoid. Three lysins (Nos. 8, 9 and 11) only dissolved bacteria from the homologous stools. Lysin No. 10 dissolved Coli B from 2 different patients.

In addition it is shewn that the lysins were not only unspecific

in respect to the species (typhoid, Coli B etc.) but that they within the various species dissolved some without dissolving others.

TECHNIQUE FOR THE DEMONSTRATION OF LYSIN.

Through these researches on the occurrence of lysin in patients with intestinal affections, I quickly suspected that d'Herelle's technique admitted of some accidental circumstances. My next aim was to acquire clarity with regard to the relation of the lysin to the bacteria, taking for granted that the dissolution of these latter must comply with definite rules in respect to time, so that a definite point of time after the filtrate had been added to the bacteria, would yield the greatest possibility of obtaining plaques when seeded on an agar plate. As this point of time possibly differed as regard various lysins, in the following experiment 4 lysins were included.

The experiment was arranged as follows: To 24 hours old cultures of 4 different bacteria (CV, T3, CB and T1) the same quantity (15 dr.) of a lysin corresponding to the bacteria was added, whereupon was seeded at the times mentioned. The experiment was carried out in duplicate. The plates with the seedings were placed in a thermostat for 24 hours and then described. At the time the experiment was made the plaques were the only certain evidence that lysin was present. If only scattered colonies grew on the plates it was not accounted a sure sign of lysin. On the following table the result is shewn in such a manner that + denotes plaques, — no plaques.

After hours:

	0	1½	3½	6	8½	10½	13	18	21½	25	30½	43½	50	54
C V.....	++	+	++	++	—	—	++	—	—	—	—	—	—	—
C V.....	+	+	+	+	++	++	++	++	++	++	++			
T III ...	—	—	++	—	—	—	—	—	—	—	—	—	—	—
T III ...	—	—	—	—	—	—	—	—	++	—	—	—	—	—
C B.....	+	+	—	—	—	—	—	—	—	—	—	—	—	—
C B.....	+	+	++	—	—	—	—	—	—	—	—	—	—	—
T I.	?	+	++	—	++	—	++	++	—	++	—	—	—	—
T I.	+	+	+	+	++	—	++	—	++	++	++	—	—	—

As will be seen the test gave no data as to the determination of the periodicity of the dissolution. The evaluation of the aspect of the plates that subsequently proved to be the right one, gave to the table the appearance shewn below. During this evaluation not only the occurrence of the plaques, but also the other aspects of the lysins are taken into consideration (more or less clean plates with varying numbers of resistant colonies).

Seeding after hours:

	0	1½	3½	6	8	10	13	18	21½	25	30½	43½	50	54
...	++	++	—	++	+++	+++	+++	+++	+++	—	—	—	—	—
...	++	++	+++	++	+++	+++	+++	+++	+++	+++	+++	—	—	—
...	+++	+++	+++	+++	—	–	+++	—	—	—	—	—	—	–
...	?	++	+++	+++	+++	+++	+++	+++	+++	—	—	—	—	—
...	++	+++	—	+++	+++	+++	+++	—	—	—	—	—	—	—
...	++	+++	+++	+++	—	+++	+++	—	—	—	—	—	—	—
...	++	+++	+++	+++	+++	+++	+++	+++	—	+++	—	—	—	—
...	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	—	—	—

The lysins according to this have acted vigorously from the very beginning and this has been almost constant until 24 hours after the lysin is added to the culture. After this juncture the lysin does not yield any visible aspect. The explanation to this will appear in another experiment.

The test-tubes with the mixture lysin-bacteria were under observation during the whole experiment at the times stated on the table. Some became a little limpid, denoting the lysis, becoming later more cloudy, others did not become limpid. From this limpidity it was, thus, not possible to draw any conclusions as to the progress of the process.

The quantity of lysin used thus proved from the very beginning to give an almost maximum effect, that, under the experimental conditions employed, remained practically unchanged for the first 24 hours. Therefore it was necessary to make experiments during which the quantity of lysin was varied. It had also proved advantageous instead of 2 hours old cultures, to use bouillon to which was added one drop of 24 hours old culture.

The following experiments were carried out according to the table below. 6 test tubes with sterile bouillon were used. To these lysin was added in the quantities stated, the total volume in all of them

being 4 c.c. To each tube 1 normal drop *) of 24 hours old culture was added. The bacteria and lysin employed were Coli CB and lysin CB respectively.

Tube No.....	1	2	3	4	5	6
lysin c.c.....	0,0005	0,008	0,128	2,48	4,00	0
coli culture..	1 dr.	1 dr.	1 dr.	1 dr.	1 dr.	1 dr.

The stated quantities of lysin are the outcome of a larger experiment in which the quantity of lysin in each successive tube is doubled.

From the 6 tubes was seeded at the times stated on small agar plates, these were placed in a thermostat for 24 hours and then described.

Tube No. 1 (0,0005 c.c. lysin).

Seeding after:

0 hours: Confluent growth with some few doubtful plaques.

1 hour: A uniformly confluent growth with numerous plaques.

$1\frac{3}{4}$ hours: Growth partially scattered, partially confluent with numerous plaques.

$2\frac{3}{4}$ - On the plate a total of 4 regular circular colonies.

$5\frac{1}{4}$ - 50 colonies, some round and regular, some "nibbled"; equal number of each.

$6\frac{1}{4}$ - About 200 colonies only a few "nibbled".

$7\frac{3}{4}$ - Plate almost covered with isolated colonies, only a few "nibbled".

$9\frac{3}{4}$ - As above.

23 - Scattered colonies on the majority of the plate, some tiny, unsaturated; some larger and more saturated.

35 - Confluent growth on the whole plate, one single doubtful plaque.

56 - Confluent growth without plaques.

$72\frac{3}{4}$ - Scattered and confluent growth of circular colonies; no plaques no "nibbled" colonies.

*) The normal drop pipette as shewn in Pharm. Dan.

$82\frac{3}{4}$ hours: Confluent growth, no plaques.
$96\frac{3}{4}$ - - - - -
$120\frac{3}{4}$ - - - - -
$143\frac{3}{4}$ - - - - -

From the seedings from this tube it will be seen that the lysin volume has been too small, immediately to produce plaques. An hour later there are numerous plaques that thereupon increase in numbers so that in the course of $2\frac{3}{1}$ hours there is a completely clean plate with but 4 resistant colonies. The resistant colonies then increase in numbers until they, after $7\frac{3}{4}$ and $9\frac{3}{4}$ hours, almost cover the plate. The following seeding carried out 23 hours after, yields an appearance almost the same as after $9\frac{3}{4}$ hours. At the subsequent seedings confluent growth without plaques was obtained.

In the bouillon tube after $23\frac{1}{2}$ hours there was not even macroscopically visible growth. This was not observed until after 35 hours had elapsed.

Tube No. 2 (0,008 c.c. lysin).

Seeding after:

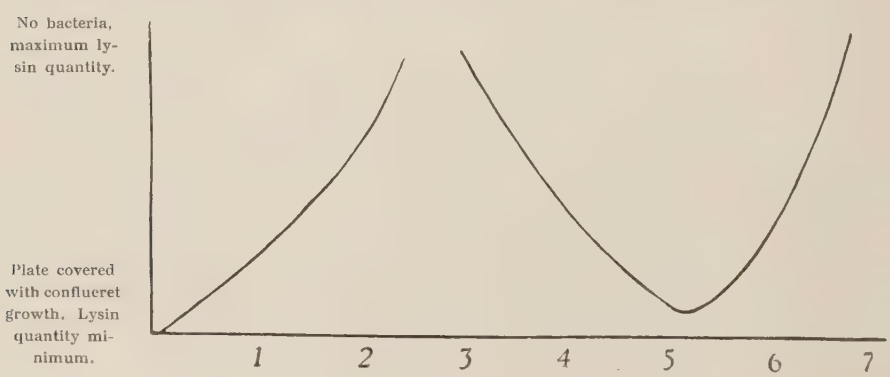
0 hours: The plate covered with confluent growth with a number of plaques.
1 hour: Plate covered with confluent growth with very numerous plaques partly confluent.
$1\frac{3}{4}$ hours: Growth consists for the most part of isolated colonies that almost all are very "nibbled". At some few spots they have run into each other forming mottled spots the size of a halfpenny. Only a few regular, circular colonies.
$2\frac{3}{4}$ - 11 large, regular, circular colonies, none nibbled.
$5\frac{1}{4}$ - 90 regular, circular colonies, none nibbled.
$6\frac{1}{4}$ - 100-200 regular, circular colonies, none nibbled.
$7\frac{3}{4}$ - Plate covered with regular, circular colonies, none nibbled.
$9\frac{3}{4}$ - Plate covered with a confluent growth.
$23\frac{1}{2}$ - Plate absolutely sterile.
$35\frac{3}{4}$ - Scattered and confluent growth, for the most part of unsaturated colonies, no plaques.
56 - Even confluent growth with very numerous plaques.
$72\frac{3}{4}$ - Scattered and confluent growth, no plaques or "nibbled" colonies.

$82\frac{3}{4}$ hours: Even confluent growth no plaques.
$96\frac{3}{4}$ - : - - - - -
$120\frac{3}{4}$ - : - - - - -
$143\frac{3}{4}$ - : - - - - -

The effect in this tube has from the commencement been somewhat stronger than in tube No. 1 and already after the lapse of an hour the plaques ran together forming bare spaces. The spreading after $1\frac{3}{4}$ hours can only be explained by the fact that a greater number of cultures were spread than on the other plates; thereupon the resistant colonies increase in number until they after $9\frac{3}{4}$ hours cover the plate. After $23\frac{1}{2}$ hours the plate is sterile. After $35\frac{1}{2}$ hours numerous resistant colonies are again found, after $56\frac{1}{4}$ hours numerous plaques on otherwise even growth; after $72\frac{3}{4}$ hours numerous resistant colonies. How are these various findings to be explained? One moment the plate is covered with isolated colonies, the next they have disappeared, and the next again the plate is found to be covered by an even confluent growth interrupted by numerous clear spots.

From other quarters attention has been called to the fact that the dissolution of the bacteria was said to occur in gusts, the lysin volume increasing simultaneously with the dissolution of the bacteria, but that when a certain point was reached, this changed so that the lysin volume decreased and the quantity of bacteria increased. This was said to be repeated several times during the process of dissolution, causing an undulatory movement.

A clear illustration is obtained if one sets down the ratio between the lysin and the bacteria on the abscissa in a co-ordinate system where the co-ordinate states the time.



If tube No. 1, for instance, is taken as an example, immediately on seeding no plaques are found: the lysin quantity is minimum, after $2\frac{3}{4}$ hours on seeding only 4 colonies are found on the plate; the number of bacteria is minimum. Thereupon the number of bacteria increases so that after 5 hours there are 50 colonies, after $6\frac{1}{4}$ hours ca. 200 and after $7\frac{3}{4}$ hours the plate is almost covered. There is now a minimum quantity of lysin and a large number of bacteria. Thereupon the development should be that lysin again increased and the number of bacteria decreased. The intervals between the seedings in this experiment are, however, too great to make an establishment of the further development possible. If we observe the seedings from tube No. 2 it is possible to determine some few points of the curve. After the 23rd hour there should be a maximum quantity of lysin and a minimum quantity of bacteria. After $56\frac{1}{4}$ hours the bacterial numbers should be decreasing and the lysin volume increasing.

Tube No. 3 (0,128 c.c. lysin).

Seeding after:

0 hours: Confluent growth with numerous plaques.

1 hour: Plate covered with isolated colonies. Some of these are very minute, some larger, very "nibbled", only about 20 large, regular, circular.

$1\frac{3}{4}$ hours: About 25 colonies of which some are small, a couple "nibbled", the rest regular, circular.

$2\frac{3}{4}$ - Only 6 regular, circular.

$5\frac{1}{4}$ - About 100 regular, circular, none "nibbled".

$6\frac{1}{4}$ - 200 colonies of similar appearance as above.

$7\frac{3}{4}$ - Plate covered for the most part by confluent colonies, no "nibbled".

$9\frac{3}{4}$ - Plate covered by confluent growth without plaques.

$23\frac{1}{2}$ - Plate covered by a very thick layer, on the thinner parts two different sorts of colonies can be distinguished: slightly saturated and very saturated.

$35\frac{1}{2}$ - Plate covered with confluent and isolated colonies, some saturated, some unsaturated.

$56\frac{1}{4}$ - Confluent growth with very numerous plaques.

$72\frac{3}{4}$ - Scattered and confluent growth of circular colonies, no plaques, no "nibbled" colonies.

$82\frac{3}{4}$ hours: confluent growth without plaques.
$96\frac{3}{4}$ - - - - -
$120\frac{3}{4}$ - - - - -
$143\frac{3}{4}$ - - - - -

The tube was quite clear during the first 23 hours, thereupon there was a steadily increasing cloudiness that persisted throughout the experiment. The volume of lysin was maximum after $2\frac{3}{4}$ hours, thereupon decreasing to minimum after $9\frac{3}{4}$ hours. At the 56th hour there was a greatly increasing lysin volume and decreasing quantity of bacteria.

Tube No. 4 (2,48 c.c. lysin).

Seeding after:

0 hours: Confluent growth, very numerous plaques.
1 hour: 75 colonies in all, 65 large, regular, circular; the remainder either very small or very "nibbled".
$1\frac{3}{1}$ hours: 6 large circular colonies.
$2\frac{3}{4}$ - 34 colonies, 2 "nibbled"; the remainder large regular, circular.
$5\frac{1}{4}$ - Plate densely covered with colonies, equal numbers of circular and "nibbled".
$6\frac{1}{4}$ - A few more colonies, otherwise as after $5\frac{1}{4}$.
$7\frac{3}{4}$ - Confluent growth without plaques.
$9\frac{3}{4}$ - - - - -
$23\frac{3}{4}$ - Confluent growth with very numerous plaques.
$35\frac{1}{2}$ - Plate covered with gelatinous greatly confluent colonies that are questionably "nibbled".
$56\frac{1}{2}$ - Confluent growth with isolated plaques.
$72\frac{3}{4}$ - Scattered and confluent growth of circular colonies; no plaques or "nibbled" colonies.
$82\frac{3}{4}$ - - - - - - - - -
$96\frac{3}{4}$ - - - - - - - - -
$120\frac{3}{4}$ - - - - - - - - -
$143\frac{3}{4}$ - - - - - - - - -

In the tube cloudiness was first observed after a lapse of 35 hours.

The maximum of the lysin was in this case reached after $1\frac{3}{4}$ hours, and between the 6th and 7th hours there is again minimum lytic

action, after $23\frac{1}{2}$ hours the lytic action again rapidly increases, in a lesser degree, however, after $56\frac{1}{4}$ hours.

Tube No. 5 (4 c.c. lysin).

Seeding after:

0 hours: Plate presents a somewhat different appearance than the others. By far the major portion is covered with tiny, densely placed colonies forming a chagreen-like background for a lesser number of saturated colonies of about the same size. In the margin are found a number of very saturated, "nibbled" colonies.

1 hour: A total of 29 colonies almost all regular circular; two only "nibbled" and burst.

$1\frac{3}{4}$ hours: 12 colonies in all, 3 "nibbled", the remainder regular circular.

$2\frac{3}{4}$ - About 25 colonies of which some are "nibbled", the remainder regular, circular.

$5\frac{1}{4}$. Some more colonies, the proportion between two kinds is the same.

$6\frac{1}{4}$ - As above.

$7\frac{3}{4}$ - Plate covered with colonies, some "nibbled", some circular.

$9\frac{3}{4}$ - Confluent growth without plaques.

$23\frac{1}{2}$ - Only a few isolated saturated colonies.

$35\frac{1}{2}$ - Plate covered with gelatinous mostly confluent colonies that are questionably "nibbled".

$56\frac{1}{4}$ - Scattered growth of tiny "nibbled" and circular colonies.

$72\frac{3}{4}$ - As above.

$82\frac{3}{4}$ - - -

$96\frac{3}{4}$ - - -

$120\frac{3}{4}$ - - -

$143\frac{3}{4}$ - - -

Macroscopically visible growth in the bouillon tube not observed until after 56 hours.

It is not easy with certainty to determine how the process has progressed in this tube, but in the course of an hour only 29 colonies grew on the plate. Not until after $5\frac{3}{4}$ hours did there again

grow a considerable number of colonies and after $9\frac{3}{4}$ hours the plate was covered by a confluent growth.

Tube No. 6 (0 c.c. lysin).

All seedings from this tube yielded confluent growth without plaques.

As is shewn by these experiments the time of the seedings was kept so far apart that one could not form a definite idea as to the course of the process. I undertook, therefore, some few experiments on the same scale, but seeded from the tubes at intervals of one hour for 56 hours. These experiments, that will not be described in detail, yielded a somewhat clearer picture of the course of the process; this, however, was not satisfactory, for the difficulties in reading the plates proved to be of greater import than primarily thought. The first seedings, in which the number of plaques increased until the plate was sterile and the next in which the number of colonies rose until the plate was covered, caused, however, no difficulties.

The first phase of the process (increase of lysin) took place within the first three hours. The greater the original concentration was, the more rapidly the maximum was reached. The next phase, in which the number of colonies increased until the plate was covered with confluent growth, took about 6 hours and was practically uniform with all initial concentrations. The subsequent seedings all shewed such varying conditions that from these it was impossible to establish any rule as to the course of the process.

It would, however, appear to be granted that constantly up to the 52nd hour an alternating action took place between the various batches of bacteria of varying susceptibility towards the action of the lysin or between bacteria on the one hand and varying quantities of lysin on the other, for until the aforesaid point of time there occurred plates both with plaques or other signs of lytic action and plates without.

As already mentioned, with the technique employed it was impossible to establish any rule for this alternating action.

These experiments were, however, of decisive significance to the technique for demonstrating lysin. If one has a fluid to be examined for lysin it should be done as mentioned, by adding a quantity of

the fluid to a suspension of bacteria and undertaking seedings from the mixture. The quantity of lysin added by this means is unknown and varies considerably (maybe 0), but the aforementioned experiments establish that only by means of seeding repeatedly for the first 3 hours is there any likelihood of observing any effect. In a subsequent chapter the technique will be described.

By means of these experiments besides obtaining the technique for the demonstration of the lysin established, one arrived at getting an extraordinary number of seedings from mixtures of lysin and cultures in all proportions and at all stages of the lytic action. This yielded an excellent insight into the macroscopically visible forms of manifestation of the action of the lysin (the appearance of the plaques, numbers, localization, the numbers and varying appearance of the resistant colonies). These circumstances will be enlarged upon in a special chapter together with a microscopic examination of the appearance of the plaques and the resistant colonies.

A problem that the experiments did not solve in a satisfactory manner was that concerning the quantitative relation between the bacteria and the lysin on the various stages of the process of dissolution.

The alternating appearance and disappearance of the plaques, together with the other lytic effects might be imagined to be brought about in two different ways. Either by the lysin increasing and decreasing in quantity whilst the bacteria constantly grew and being, as they multiplied, gradually dissolved by the varying quantity of lysin. Or by the quantity of lysin increasing until a maximum was reached, but that the growing bacteria presented alternating susceptibility to the lytic action.

This could only be decided by means of quantitative determination of the lysin concentration and a simultaneous count of the bacteria present in the fluid. In order to undertake such it would be necessary to have a practicable method of measuring to determine the lysin concentration. In a subsequent chapter such a method will be mentioned.

THE OPTIMUM CONDITIONS FOR LYSIN.

The circumstances of main interest in this connection to be investigated were the following:

What substratum is most suitable for experiments on the lytic action?

At what temperature and reaction (p_H-value) does the lysin attain its maximum effect?

The following substrata were examined: Ordinary bouillon, aqueous agar, blood agar, ascitic agar, ordinary peptone bouillon, ascites bouillon and ascites-citrate-glucose bouillon.

In respect to the solid substrata the experiments were undertaken by spreading from the same mixture of lysin and bacteria simultaneously on the substrata mentioned, thereupon placing same in a thermostat at 37° for 24 hours, whereupon they were compared.

With the exception of aqueous agar the effect was the same on all the solid substrata in the sense that the number and size of the plaques was identical. On ascites and blood agar the bacterial growth (coli and dysentery), however, was more luxurient than on ordinary agar and the holes were consequently deeper on these substrata. On aqueous agar the growth was so sparse that this substratum can be disregarded, at any rate in a case where experiments within 24 hours are concerned.

With regard to the fluid substrata the experiments were made by seeding with equal quantities of bacterial culture and lysin test tubes containing equal quantities of various substrata and placing at 37°. From each glass was spread on agar at intervals of half an hour and the spreadings were compared after having been placed in a thermostat at 37° for 24 hours. The difference in these substrata was not so great as to occasion the use of other than ordinary peptone bouillon.

A factor that subsequently proved to play a rôle was whether the bouillon was sterilized a. m. Koch (heated at 100° in streaming steam for 30 minutes) or by autoclaving. It was namely observed that with some lysins a better result was obtained by means of the former mode of sterilisation, and this was thus introduced as the normal

method, both for bouillon as such and for the bouillon used in the making of agar plates.

The question as to what temperature was the most suitable for the lysin was complicated, in that it was not in advance granted that a temperature suitable for a reaction p_H-figure was also optimum in other reactions, even though these were within a sphere that empirically had yielded good results from the lysin. This was taken into consideration in the experiments by introducing several tubes with varying reactions instead of a single tube at each temperature.

The experiments that all were carried out with the same lysin and bacteria (coli CB) were arranged in the following manner:

The temperature in a series of water-baths was adjusted to 10^0; 20^0, 30^0, 40^0, 45^0 Celsius. In each water-bath were placed 6 test tubes with 10 c. c bouillon in each. The p_H-figures in the 6 test tubes were: 9.24, 8.73, 8.58, 8.18, 7.23, 6.72. All test tubes were seeded with 2 drops of 24 hours old culture of Coli CB, and to each tube was added the same quantity of lysin. This was so calculated that by the seeding one obtained one or some few plaques. From all tubes were seeded on agar segments of equal size at intervals of $\frac{1}{2}$ to 1 hour for 8 hours. The agar plates with the seedings were placed in a thermostat at 37^0 for 24 hours, when the plates were described.

It was then demonstrated that the variations in the p_H-figures played no significant rôle for the 6 tubes in the same water-bath yielded, in the main, identical results. The experimental results could therefore be read from the effects of the temperature alone the following being ascertained.

At 10^0: The lytic action after 8 hours not greater than at beginning of experiment: in both cases some few plaques only.

20^0: There was a considerable increase in the number of plaques; after 8 hours, however, only 15-20 plaques.

25^0: At the beginning of experiment: one single plaque.
1 hour later: 3 plaques.
$2\frac{3}{4}$ hours later: 2-300 plaques.
At subsequent seedings increasing lytic action.

30^0: At the beginning of experiment: one single plaque.
1 hour later: about 150 plaques.

$2\frac{3}{4}$ hours later: plate bare with resistant colonies.

37°: At the beginning of experiment: one single plaque.

1 hour later: plate bare with resistant colonies.

40°: At the beginning of experiment: one single plaque.

1 hour later: plate bare with resistant colonies.

45°: At beginning of experiment: one single plaque.

After one hour and later: no plaques.

It is apparent from these experiments that the most suitable temperature is between 37° and 40°. The lytic effect at temperatures over 40° decreases rapidly with the rise in temperature, there being absolutely no effect at 45°, whereas the effect at a decreasing temperature is maintained somewhat longer, it being recorded that as low as 20° there is an increase in the number of plaques, though slight.

The experiments given below in which a lower initial lysin quantity is used show this still more distinctly.

At 10°: After $5\frac{1}{4}$ hours still no plaques.

20°: At beginning of experiment: no plaques.

$5\frac{1}{2}$ hours later: 1 plaque.

25°: At beginning of experiment: no plaques.

$2\frac{3}{4}$ hours later: 1 plaque.

$3\frac{1}{2}$ - - 4 plaques.

$5\frac{1}{2}$ - - some plaques.

30°: At beginning of experiment: no plaque.

$1\frac{3}{4}$ hours later: 1 plaque

$2\frac{3}{4}$ - - numerous plaques.

$3\frac{1}{2}$ - - - -

$5\frac{1}{2}$ - - - -

37°: At beginning of experiment: no plaques.

$\frac{3}{4}$ hour later: no plaques.

$2\frac{3}{4}$ hours later: bare plate with resistant colonies.

$3\frac{1}{2}$ and $5\frac{1}{2}$ hours later; bare plate with resistant colonies.

40°: As at 37°.

45°: No lytic effect after $5\frac{1}{2}$ hours.

The optimum temperature was thus between 37°-40°. As to the reason why this should be the case the experiment gave no information. It might be imagined to be due to the optimum of lysin being at that temperature in which the bacteria best multiplied; on the

other hand it might be that the lysin had its own optimum regardless of that of the bacterial growth. This question, that is of considerable theoretical interest, was, however, outside the immediate scope of my investigations. Researches on a lysin capable of dissolving bacteria with varying growth optimum, or the cultivation for lengthy periods of those bacteria the lysin was capable of dissolving at various temperatures thereby altering their growth optimum, might aid in the solving of this problem.

From this I turned to the investigation regarding at what reaction in the substratum (p_H-figure) the lysin exercised its greatest effect.

The first experiment was made by spreading from the same mixture of lysin and bacteria on agar plates with varying reaction. The advantage of this method was that by placing the same quantity of the mixture on plates with varying reaction the optimum could be found merely by comparing the plates.

The method presented, however, innumerable difficulties. By means of numerous experiments I was successful in discovering a method for the colorimetric measuring of the reaction in bouillon-agar. The electrometric method had to be immediately rejected for obvious reasons.

(O. Henriques has subsequently afforded an easy and accurate method for the measurement of the reaction in solid substrata).

If the same quantity of a mixture of lysin and culture was seeded on a series of plates with varying reaction, the same number of plaques was invariably obtained; the size of the plaques, however, varied greatly all according to the reaction of the substratum.

If one then could take for granted that the size of the plaques solely depended upon the various reactions the method would have been practicable, but this assumption, however, proved to be erroneous. The reason for this was that the agar at the various reactions was of varying consistency and degrees of moisture, and these two factors have no little influence on the size of the plaques. To this be added the circumstance that the plates, when but slightly alkaline during the experiment became less alkaline in the most superficial layer of the agar, whereby the experimental conditions were altered. Therefore this method had to be given up an fluid substrata of varying reaction used instead.

The technique in the production of these substrata was as follows: 16 litres of bouillon was prepared in the usual manner with 1 % peptone and $^1/_2$ % NaCl. By means of 5 normal NaOH the reaction was increased to a p_H figure of ca. 10 thereby causing a considerable precipitation. It is then heated to 60° for $^1/_2$ an hour and cooled for a night in a refrigerator. The following day it is filtered until completely limpid and the reaction by means of 5 normal of lactic acid is reduced to a p_H figure of between 6 and 7. The 16 litres are divided into 500 c. c. in flasks an these are sterilized a. m. Koch 3 times at intervals of 1 day. The reason that the reaction is reduced from 10 to ca. 6 is that the bouillon cannot well stand the sterilization when it is considerably alkaline. A small quantity of the bouillon is titrated with $^1/_{10}$ normal NaOH and $^1/_{10}$ normal lactic acid to the highest p_H figures required. The titrate figures are drawn on a curve upon which it is later possible to read off what quantities of $^1/_{10}$ normal NaOH and lactic acid are to be added to obtain reactions, with the desired interval. These figures are reduced to 5 normal for 500 c. c. and the qantities resulting are added to the flasks containing the bouillon. The bouillon was then tapped into sterile test tubes, 10 c. c. in each. The reaction in 2 tubes of each series was measured both at room-temperature and at 37°. All measurings were carried out colorimetrically. The measurements at 37° were made in a water-bath with glass walls in which a Walpole comparator of lead was placed.

As demonstrated by Walbum, the bouillon, alters its reaction with the temperature. This change is, as regards bouillon with the usually employed p_H figures, but slight (at a p_H of 7 it amounts to 0.008 per 1 degree), but with the more alkaline bouillons it is considerable (at a p_H figure of 11, it is 0.33).

The prepared series of bouillon tubes represented 26 different p_H figures, from 4.50 to 11 measured at room-temperature. In the following experiments only about every other one is stated. p_H figures given are measured both at 37° and at room-temperature.

The experiment itself is arranged in the following manner: One tube of each series, a total of 26 are placed in a water-bath at 37°. When the bouillon has arrived at the temperature of the water-bath,

from a concentrated suspension of agar culture the same number of drops is added to all tubes with a normal dropping pipette so that a density corresponding to ca. standard 8 is obtained (Wulff). Thereupon is added one normal drop of lysin diluted in such a manner that by seeding a loop-ful of the mixture lysin an culture, either some few plaques or none are obtained.

From all tubes on equally large agar areas is seeded at the times stated. All seedings are carried out with the same platinum loop. The agar plates are placed in a thermostat at 37° and the following day the plaques are counted.

Eksperiment I.

B. Dys. Shiga.
Hours: Lysin: S I. diluted $^1/_{5000}$.

pH at 37°	0	1	1½	2	2½	3	4	4½	5½	pH at room-temperature
4.50	0	2	0	0	Sp.g	Sp.g	Sp.g	Sp.g	— g	4.50
5.00	0	0	0	1	1	0	0	0	0	5.00
5.50	1	0	0	0	1	1	0	0	0	5.50
6.15	1	0	4	1	6	78	230	150	75	6.20
6.56	2	1	18	29	18	36	85	97	80	6.72
6.90	0	3	53	46	68	50	28	110	weep.	7.08
7.50	0	1	29	42	31	50	150	120	130	7.64
8.16	0	2	1	4	8	12	34	50	90	8.39
8.60	1	1	0	0	0	1				8.87
8.94	0	0	1	2	0	0				9.28
9.54	0	0	0	0	0	0				9.90
10.00	Sp.g	— g	— g	— g	— g	— g	— g	— g	— g	10.40
10.30	Sp.g	— g	— g	— g	— g	— g	— g	— g	— g	11.00

The experiment shews that the optimum lies at a p_H figure of between 7 and 8.

Figures shew No. of plaques
Sp.g denotes sparse growth
— g - no -

Weep. designates that the plate has not been sufficiently dried and has therefore exuded moisture.

Experiment II.

B. Typhosus T 3.
Lysin: T 3, diluted $^1/_{2500}$.

Hours:

pH at 37°	0	$\frac{3}{4}$	$1\frac{1}{2}$	$2\frac{1}{2}$	$3\frac{1}{2}$	4	5	$5\frac{1}{2}$	pH at room-temperature
4.50	—	—	Sp.g	Sp.g	Sp.g	— g	— g	— g	4.50
5.00	—	—	—	—	—	—	—	—	5.00
5.50	—	—	—	—	—	—	—	—	5.50
6.15	—	8	—	3	—	—	3	3	6.20
6.56	—	—	—	7	34	9	40	50	6.72
6.90	—	3	weep.	21	41	160	++	+	7.08
7.50	—	7	24	26	26	125	+	+	7.64
8.16	—	2	8	12	5	23	50	125	8.39
8.60	—	—	6	1	—	6	20	30	8.87
8.94	—	—	—	—	—	—	—	—	9.28
9.54	—	—	—	—	—	—	—	—	9.90
10.00	—	—	—	— g	— g	— g	— g	— g	10,40
10.30	—	—	—	— g	— g	— g	— g	— g	11.00

The experiment shews the same optimum as experiment I.
++: plaques confluent.

Experiment III.

B. Dys. Shiga.
Lysin: Spn. diluted $^1/_{500}$.

Hours:

pH at 37°	0	$1\frac{3}{4}$	$2\frac{3}{4}$	$3\frac{3}{4}$	$4\frac{3}{4}$	pH a room-temperature
4.50	6	1	0	Sp.g	— g	4.50
5.00	5	1	2	—	0	5.00
5.50	8	1	1	—	—	5.50
6.15	10	74	+	++	+++	6.20
6.56	7	70	+	++	+++	6.72
6.90	5	350	+	++	+++	7.08
7.50	10	175	+	++	+++	7.64
8.16	2	93	204	++	++	8.39
8.60	6	—	7	1	—	8.87
8.94	5	—	—	—	—	9.20
9.54	1	2	0	0	0	9.90
10.00	4	Sp.g	Sp.g	Sp.g	— g	10.40
10.30						11.00

The experiment shewed the same optimum as in the two previous experiments.

Experiment IV.

Coli C.B.
Lysin: C.B.

Hours:							
pH at 37°	0	1½	2½	3	3¾	4¾	pH at room-temperature
4.50	0	1	1	0	0	0	4.50
5.00	0	0	0	0	0	0	5.00
5.50	0	0	0	1	0	0	5.50
6.15	0	1	3	50	51		6.20
6.56	3	4	45	10	60		6.72
6.90	1	49	60	+	++		7.08
7.50	0	32	+	++	+++	++++	7.64
8.16	0	40	+	++	+++	++++	8.39
8.60	3	40	92	+	++	+++	8.87
8.94	0	16	76	+	+	++	9.28
9.50	0	0	0	50	(+)	(+)	9.90
10.00	0	0	0	0	0	0	10.40
10.30	0	Sp.g	Sp.g	Sp.g	— g	— g	11.00

The experiment shewed, that the optimum reaction lay between p_H 6.90 and p_H 8.60.

These experiments of which, besides those stated, a number of others were undertaken, demonstrate that an optimum for the bacteria and lysins examined lies between p_H 7 and p_H 8, in respect to the coli strain examined, perhaps slightly higher.

Furthermore it is observed that at p_H figures of ca. 6 and up to p_H figures of about 8, no noteworthy increase of lysin is obtained in the course of 5 hours.

At p_H figures of 4—5 and above 10 bacterial growth is obtained at first seeding, but at the subsequent passages there is no growth at all. This goes to shew that the bacteria with the reaction in question and at a temperature of 37° are killed within 1 to 4 hours.

In respect to the reaction optimum the same views must be held as stated in the researches on the optimum of temperature. Nothing is known as to whether the lysin possesses its own optimum or whether its optimum is associated with the growth optimum of the bacterium in question.

MEASURING METHODS.

The capacity of a lysin to dissolve a bacterial culture depends upon various different properties in the lysin. It is a collective ex-

pression for these properties when we say that it is »potent«, »strong« nay we frequently see the term »virulent« used. This latter expression is so closely associated with the representation of a living virus that for this reason alone it is infelicitous. Virulence, moreover, is something that is difficult to measure.

The properties in the lysin that collectively yield an expression of its potency cannot be measured »en bloc«. Therefore it is necessary to state the potency of a lysin by means of several figures or by the proportion between these.

Should we peruse the literature regarding this subject we shall find that, as a rule, it is only one isolated property in the lysin that is given as a measure of its potency. Before enumerating the measuring methods employed, it would therefore be useful to observe more closely what properties in the lysin can be measured, and by means of this be used as an expression of its potency.

Should we mix a fluid bacterial culture with a certain quantity of a lysin capable of dissolving the bacteria in question, and if some of the mixture is seeded on solid substratum, in the resultant culture we shall obtain a greater or lesser number of plaques. A suitable expression for this circumstance would be to say that the lysin contained a certain number of plaque-producing units.

The number of plaque-producing units is the gauge that is most frequently given as a measure of the capacity of the lysin to dissolve a bacterial culture; but actually it states no more than that a certain number of units are present in the fluid in question. As to the capacity of these units to dissolve bacteria nothing is said, for this may very well vary in respect to two lysins yielding the same number of plaques. In order to express the collective effect of the units it is necessary, not only to know their numbers but also their capacity to dissolve bacteria, or in other words the time one unit takes to dissolve a certain number of bacteria.

As the lysin, however, dissolves living bacteria only and as there, at every juncture during a progressive dissolution, are a certain number of living bacteria capable of multiplying in an incalculable manner, neither the number of dissolved or remaining bacteria can be employed for estimating the lysin.

By dissolving the bacteria the lysin, as is known, increases in

quantity and in the velocity at which this increase takes place we have a gauge for the capacity of the individual units to dissolve the bacteria.

Besides the number of plaque-producing units and the velocity at which they increase we can measure the greatest number of units that can be attained by the lysin in question.

Thus should we know the number of plaque-producing units, the velocity at which they are able to increase, and the greatest number per c. c. they can attain, we possess a satisfactory illustration of the capacity to dissolve bacteria of the lysin in question.

It would be to the point to intruduce the following terms for the above-mentioned properties in the lysin:

Reaction figure: The number of plaque-producing units contained in 1 c. c. of the solution.

Reaction velocity: The time taken before the maximum number of plaque-producing units is attained, when to 200 c. c. bouillon one loop-ful of 24 hours old bacterial culture is added together with such a quantity of lysin that by the seeding of a loop-ful no plaques are produced. During the reaction the mixture is placed at 37°.

Reaction maximum: The greatest number of plaque-producing units per c. c. the lysin is capable of attaining.

On perusing the literature I have not been successful in discovering determinations of the two latter quantities. The potency of a lysin is invariably given by its contents of bacteriophages per c. c. i. e. the reaction figure. This is determined by the various authors in different ways and from various quarters erroneous sources more or less significant are given.

The first method for the measuring of lysins is given by d'Herelle. He dilutes the solution, the potency of which he wishes to measure, in a known proportion, adds the bacteria and seeds a definite quantity on agar, thereupon counting the plaques subsequently produced. By multiplication with the degree of dilution he obtains the contents per c. c.

This is also a main principle in the methods of all subsequent authors, even if, in varying ways, these deviate somewhat.

In the following an evaluation of the various methods and their sources of error will be attempted, together with a description of a

technique that with the greatest degree of accuracy and a minimum of work, permits of the determination of the reaction figure.

In none of the methods avanced has it been possible to avoid a dilution of the fluid, the contents of plaque-producing units of which is to be measured. Here one encounters the same sources of error, that are apparent in every dilution. Doerr and Grüninger emphasize especially the pipette error, i. e. that a certain quantity of the original solution remains in the pipette throughout more dilutions than calculated. In the dilution of lysins the circumstances in this respect are the same as in the dilution of bacterial cultures. That this may be fatal when working with units capable of multiplication is obvious. This error is avoided by changing the pipette at each dilution, and may be restricted by arranging ones technique in such a manner that the units in question are not given the occasion of multiplying before they are isolated.

The dilution may be carried out with bouillon or physiological saline solution and the bacteria added to the prepared dilutions, or a bacillar suspension in bouillon or physiological saline solution may be used for the dilution. As the bacilli adhere more closely to the pipette than the lysin units, the use of bacillar suspension as diluent should yield greater pipette erors.

If, when the dilutions are prepared, their contents of plaque-producing units are to be determined, opinions differ as to how this can be carried out with the greatest accuracy.

Appelmanns, Wertemann, Doerr and Grüninger and others prepared the dilutions in volumes of 10 c. c., so that each tube contained $^1/_{10}$ of the lysin contained in the preceeding one and placed in each tube a loopful of 16 hours old culture, whereupon all the tubes were placed in the thermostat until the following day. It will then be seen that the tubes with the highest concentrations have remained limpid whereas those containing a lower concentration have become cloudy expressing the fact that they have not contained lysin. The last tube in which full limpidity is recorded is then regarded as containing one bacteriophage unit. If the degree of dilution in the tubes is, for instance, 1 to 10^{-3} the lysin exponent is said to be 3. The potency of the lysin is thus given by the degree of dilution expressed by the negative exponent to 10. This method

is, as a rule, termed the dilution method as opposed to the seeding method, that subsequently will be described.

Personal experiments have, however, shewn me that it is not rational, the reasons given being as follows:

In respect to many lysins, even with high concentrations, one does not attain complete dissolution. Again it is quite subjective what is understood by complete dissolution, one observer being capable of recording a cloudiness that another fails to see. The two following experiments, however, prove decisively that the method is impracticable.

To 10 c. c. of bouillon is added a loopful of 24 hours old culture and the mixture is shaken. This does not cause any visible cloudiness in the tube. Thereupon a quantity of lysin is added, so calculated that on seeding a loopful some few plaques will be obtained. The tube is placed at 37^0 and every 15 minutes seedings are undertaken. The tube is examined every $^1/_4$ of and hour an it is noted that no visible cloudiness appears for several hours. The following day the seedings shew that the number of plaques has increased every $^1/_4$ of an hour until finally there was no growth whatever on the plates.

The second experiment is carried out in the same manner as the first, with the sole difference that, to the tube is added so much of the culture that a distinct cloudiness appears. On observing this tube it will be noted that it remains cloudy; maybe the cloudiness decreases somewhat during the first few hours, but later it again increases, the seedings on agar shew exactly the same proportions as the seedings from the first tube.

The first experiment shews that in a tube in which there is no macroscopically visible cloudiness, may be found from one single plaque-producing unit to a great number. The second experiment shews that the same may be the case in a very cloudy tube.

The fact that the tube has remained clear or has become cloudy cannot therefore be used as a quantitative estimation the volume of lysin. The only case where such a limpidity or cloudiness would be decisive is where we encounter a lysin, the lytic capacity of which is absolute, in the sense that one single plaque-producing unit is

capable of dissolving the bacteria without a secondary growth of resistant bacteria.

Every method that is merely based on the clearing up as a criterium for the presence of lysin must consequently be regarded as unsuitable for the accurate measuring of the number of plaque-producing units.

In this connection may be mentioned that Beckerich and Hauduroy recommend both the dilution and seeding methods separately. The dilution method should be employed with potent lysins, these yielding by the seeding method too high values. The seeding method should be used with weak lysins, these yielding too low values by the dilution method. I have not observed anything similar. Possibly this may be explained by the fact that the pipette error has not been taken into consideration. As will be seen by the following this circumstance does not arrive at playing any rôle whatever in the technique advanced by me.

The dilutions having been prepared the examination of these may be undertaken in various ways. The majority of authors add the bacteria to the dilutions and seed from the mixture. Gratia emphasizes that the bacterial concentration is not a matter of indifference in respect to the number of plaques, whereas d'Herelle is of the opinion that this remains the same whether many or few bacteria are added. This question being of signal importance as to the accuracy of the method I have undertaken some experiments in this direction. Of these I shall merely report a couple.

From an agar culture a very dense suspension of bacilli was prepared, this was diluted in the following proportions: $^1/_{500}$, $^1/_{250}$, $^1/_{100}$ $^1/_{50}$, $^1/_{25}$, $^1/_{10}$ and $^1/_5$. A dilution of lysin was prepared that, mixed with the bacteria, yielded on the seeding of one loopful an easily countable number of plaques. This dilution was divided into 12 dwarf test-tubes 3 c. c. in each. To these 12 test-tubes dilutions of bacilli according to the following table were added and after mixing thoroughly was seeded on agar plates from all the tubes, using the same loop and seeding on agar areas of the same size.

Experiment No. I. (Paratyphoid 1022).

Tube No.	1 drop of dilution of culture		$^1/_{500}$ + 3 cc. dilution of lysin		15 plaques
2	1	—	$^1/_{250}$ + 3	—	23 —
3	1	—	$^1/_{100}$ + 3	—	35 —
4	1	—	$^1/_{50}$ + 3	—	30 —
5	1	—	$^1/_{25}$ + 3	—	41 —
6	1	—	$^1/_{10}$ + 3	—	85 —
7	1	—	$^1/_{5}$ + 3	—	68 —
8	1	undiluted culture	+ 3	—	125 —
9	2	—	+ 3	—	99 —
10	4	—	+ 3	—	104 —
11	8	—	+ 3	—	39 —
12	16	—	+ 3	—	18 —

In another experiment arranged according to the same table shewed the following figures:

Experiment No. II. (Paratyphoid 1334)

Tube No.	1 : 0 plaques
2	: 0 -
3	: 0 -
4	: 7 -
5	: 7 -
6	: 26 -
7	: 20 -
8	: 26 -
9	: 26 -
10	: 18 -
11	: 4 -
12	: 3 -

In both these experiments the density of tube No. 9 was as standard 8 (Wulff) and it decreased as is shewn on the table by one half in each tube of a lower number, whereas it was doubled in each tube bearing a higher number. In the tubes from 9—12 an error in dilution occurs that in the tube in which it is greatest, No. 12, does not exceed 25 %.

It is evident from this that the number of the plaques increases with a rising bacilli concentration until an optimum is reached that attains a density of about standard 8 (Wulff) whereupon they decrease. The same circumstances were observed by Gratia.

After having added culture in such a quantity that the required concentration is obtained, the agar must be seeded. This seeding

should invariably be carried out as uniformly as possible with regard to the manner in which the seeding is done and the quantity seeded and also in respect to the agar area upon which is seeded.

Originally I used a normal drop pipette for depositing a drop on the agar plate and I then spread it out with a goose-neck glass rod. This yielded extremely heterogenous results, presumably due to the fact that on the various glass rods varying quantities of the drop remained adhering. Otto and Munter have suggested depositing a drop of the fluid to be examined on agar that is seeded with bacteria without spreading out the drop. As this prevents the counting of the plaques, in my method it is without significance. I have consequently chosen to spread with one and the same platinum loop by all measurements. The loop is dipped entirely in fluid and lifted out in exactly the same manner i. e. with the plane of the loop parallel with the surface of the fluid. The quantity of fluid contained in the loop was thereupon distributed evenly upon $^1/_8$ of the surface of a large agar plate, smoothing it over the whole area until both this and loop were dry. If one stops before this is accomplished an uneven distribution of the plaques is easily obtained and moreover there is a possibility that the plaque-producing units in the fluid are increased, whereby the result becomes inaccurate.

The agar used must possess the optimum hydrogen ion concentration for the lysin which is, already mentioned, at a p_H figure of about 8. The agar plates should be well dried for a possible exudation absolute compromises the results, owing to the fact that number of the plaque-producing units in the exuded fluid increase rapidly. This as a rule makes itself evident by the plaques running together and forming large spots thus preventing the counting of them; but the formation of several scattered plaques corresponding to the contents of plaque-producing units, cannot likewise be excluded.

After being spread upon agar plates, these are placed in a thermostat and the following day the plaques are counted.

The counting of the plaques occasions, as a rule, no difficulty. They may be of varying sizes and, should they be too small for the naked eye, a magnifying glass is used.

It frequently occurs that the size of the plaques varies within the same seeding, likewise the plaques are often surrounded by a zone

that has not been fully cleared up. At times this zone may be just as broad as the plaque itself. As I am not of the opinion that the size of the plaques indicates whether one or several plaque-producing units have lain in the same spot and as it is merely a question of determining the number, the lytic capacity of the individual units being determined by other means, I have disregarded this difference in size. It is granted that a plaque may become larger through several plaque-producing units lying side by side, but this, as a rule, will invariably evidence itself in the shape of the plaques becoming irregular.

Before proceeding to describe the technique used by me in the counting of the plaque-producing units I shall briefly report on a couple of methods that in principle deviate from those hitherto mentioned. Elizabeth Leppert sees clearly that the measure of the capacity of a lysin to dissolve bacteria, is but insufficiently expressed by stating the number of plaques; she lays especial stress on the fact that the plaques frequently are surrounded by the aforementioned zone. Therefore she measures the lytic capacity by comparing the action of the lysin with the killing power of various phenol solutions against the bacteria by counting the surviving bacteria. That this is erroneous is evident from the previously stated argument that in a mixture lysin-bacteria there will always be bacteria that are developing, which fact prevents the determination of the number of the surviving.

Personally I endeavoured in my first experiments to elaborate a counting method to seed the mixture lysin-bacteria in fluid substratum. The experiment was carried out by preparing a series of known dilutions of the lysin in bouillon with the same volume in all test-tubes. To these tubes as also to a tube without lysin the same quantity of a bacterial culture was added. This was calculated in such a manner that on the seeding of a definite quantity in 20 c. c. fluid agar an easily countable number of colonies was obtained. I thereupon expected that the number of colonies in the seedings from the tubes containing the greatest quantity of lysin would be reduced in proportion to the seedings from those containing less lysin.

I was, however, disappointed in my expectations, finding as I did about the same number of colonies in all the seedings. On surface colonies only, some difference was observed, produced by the various

concentrations of lysin. In the seedings from the tubes containing the highest concentrations of lysin, almost all the surface colonies were attacked by the lysin, there being perforated, nibbled or only insignificant remains of them to be seen. The number of attacked colonies decreased with the falling lysin concentration. The decisive factor, however, was that the colonies down in the congealed agar were never found to have been attacked. This might be due to two circumstances, viz. either the lysin was not capable of exercising any action under wholly or partially anaërobic conditions, or else the bacteria were invariably separated from the lysin units by a layer of agar. The former possibility proved by superficial seedings, cultivated under anaërobic conditions, not to be the case, for the lysin here proved to have the same effect as under aërobic conditions, the latter possibility may thus be regarded as being correct.

I consider it very improbable that it was the antilytic action in the agar as discribed by Doerr and Berger to which the phenomenon was due. They state, namely, that the antilytic action of the agar is only capable of inhibiting the action of very weak lysins, whilst it cannot assert itself against fairly potent concentrations of lysin. The above-mentioned phenomenon moreover occurs, also when potent lysins are used undiluted.

The technique I have employed in counting the number of plaque-producing units is, in the main, the same as demonstrated by Walbum for the counting of bacteria, seedings on agar plates being substituted for seedings in fluid agar.

In each of a series of sterile test-tubes 2.7 c. c. bouillon is measured, in the first one with a sterile normal drop-pipette, 6 drops of the lysin to be measured is deposited and the tube is carefully shaken, whereupon with a fresh drop pipette from this tube 6 drops are transferred to the next and so on, until a dilution has been attained in which there cannot be presumed to be plaque-producing units. By this means a series of dilutions is obtained, in which each tube contains ten times as little lysin as the preceding one. The day before a large agar plate has been seeded with the bacteria against which the action of the lysin is to be measured. The culture on this agar plate is suspended in a small quantity of bouillon and the suspension is diluted so considerably, that one drop added to 2.7 c. c.

bouillon yields a density corresponding to ca. standard 8 (Wulff). A drop of culture dilution is now added to each of the prepared dilutions and after careful mixing it is spread on agar. The spreading is undertaken with a platinum loop. Its contents are once and for all determined by repeated weighing of the loop both with its contents and after has been smoothed dry on the agar plate. As already said, the loop is filled by dipping it into the fluid and lifting it up with the plane of the loop parallel with the surface of the fluid. The seedings are carried out on the $^1/_8$ of a large agar plate and one continues to smooth the loop backwards and forwards over the area until both this and the loop are dry, which does not take long when the agar plate beforehand is well dried. The agar plates are placed at 37° until the following day.

If one has used lysin with a considerable content of plaque-producing units, one will note that the plaques from the seedings of the first tube have run together forming bare areas, but little by little as the seedings from the weaker lysin dilutions are reached, this is the case in a lesser degree and at the first seeding in which the plaques are isolated the count begins. Likewise, in the subsequent seedings, the plaques are counted until one is reached that produces none.

As an example some counts will be given.

1. Coli Lanz. Lysin Lanz.

Simultaneously 10 parallel experiments were carried out independently.

Tube No.	1	2	3	4	5	6	7
Degree of dilution	$^1/_{10}$	$^1/_{100}$	$^1/_{1000}$	$^1/_{10000}$	$^1/_{100000}$	$^1/_{1000000}$	$^1/_{10000000}$
	+	+	+	+	127	15	1
	+	+	+	+	91	7	2
	+	+	+	+	84	9	0
	+	+	+	+	88	4	1
	+	+	+	+	135	14	1
	+	+	+	+	65	6	1
	+	+	+	+	81	6	1
	+	+	+	+	118	17	0
	+	+	+	+	107	9	0

The calculation of the number of plaque-producing units is best illustrated by an example.

In one experiment the following figures resulted:

Degree of dilution:	$^1/_{10}$	$^1/_{100}$	$^1/_{1000}$	$^1/_{10000}$	$^1/_{100000}$	$^1/_{1000000}$
No. of plaques:	+	+	+	135	14	1

If 14 is multiplied by 10 one arrives at the number of plaques corresponding to a dilution $^1/_{10000}$, viz. 140, whereas the figure found is 135. The average between these is the number of plaque-producing units to be found in a loopful of the dilution $^1/_{10000}$. By multiplying by 10000 we get the number found in a loopful of the undiluted lysin. The loop contains 0.0056 c. c. By multiplying by 10000 and dividing by 56 the number of plaque-producing units per c. c. is obtained.

$$\frac{137.5 \cdot 10000 \cdot 10000}{56} = 24553000$$

Coli d'H. Lysin d'H.

12 parallel measurements were made.

Tube No.	1	2	3	4	5	6	7
Degree of dilution	$^1/_{10}$	$^1/_{100}$	$^1/_{1000}$	$^1/_{10000}$	$^1/_{100000}$	$^1/_{1000000}$	$^1/_{10000000}$
	+	252	24	2	0	0	0
	+	276	20	1	0	0	0
	+	100+	10	0	0	0	0
	+	231	20	1	0	0	0
	+	205	21	2	0	0	0
	+	213	19	3	0	0	0
	+	135	12	1	0	0	0
	+	203	32	0	0	0	0
	+	206	16	1	0	0	0
	+	236	28	4	0	0	0

Coli Tietz. Lysin Tietz.

Tube No.	1	2	3	4	5	6	7
Degree of dilution	$^1/_{10}$	$^1/_{100}$	$^1/_{1000}$	$^1/_{10000}$	$^1/_{100000}$	$^1/_{1000000}$	$^1/_{10000000}$
	+	+	+	+	92	13	1
	+	+	+	+	97	9	0
	+	+	+	+	79	7	0
	+	+	+	+	56	6	0
	+	+	+	+	54	5	0
	+	+	+	+	76	7	0
	+	+	+	+	56	4	0

Dysentery Shiga. Lysin S I.

Tube No.	1	2	3	4	5	6	7
Degree of dilution	1/10	1/100	1/1000	1/10000	1/100000	1/1000000	1/10000000
	39	5	0	0	0	0	0
	36	4	0	0	0	0	0
	39	4	0	0	0	0	0
	40	3	0	0	0	0	0
	42	5	0	0	0	0	0
	36	4	0	0	0	0	0
	66	8	0	0	0	0	0
	61	6	0	0	0	0	0
	41	5	0	0	0	0	0
			Plate has wept				

As will be seen by the above parallel counts the method possesses an accuracy sufficient for ordinary bacteriological purposes.

CONTINUED EXPERIMENTS ON THE COURSE OF THE PROCESS OF DISSOLUTION.

As mentioned on page 76, the separate phases in the process of dissolution could only be elucidated by a quantitative analysis by which both the lysin concentration and the concentration of bacilli at the various stages of the process was determined.

Keeping in view this object a number of experiments were undertaken during which, at short intervals, specimens of a mixture of lysin and bacteria placed at 37° were taken. In the specimens taken the number of living bacteria and the number of plaque-producing units was determined after the bacteria had been killed by heat.

The technique was as follows: 200 c.c. of bouillon in a flask of 300 c.c. is heated to 37°, a previously calculated quantity of lysin is added, yielding such a lysin concentration that by seeding a loop-ful, one single or no plaques are obtained. To this 5 normal drops of a 24 hours old culture is added. At the intervals determined, specimens of 2 c.c. are taken with a sterile Pasteur pipette. One c.c. of the specimen taken is placed in a small sterile glass ampule that is immediately sealed and deposited in a water bath at 56°, where it remains for 1/2 an hour. By this means the bacteria are killed

without affecting the lysin. That this was the case, was ascertained by experiments on the thermal death-point of the lysin. Later by means of the previously mentioned technique the number of plaque-producing units was determined in all the specimens of this description.

The remainder of the specimens taken were used for determining the number of living bacteria a. m. Walbum. Walbum's technique is briefly as follows: In each of 7 sterile test tubes 2.7 c. c. sterile saline solution is deposited with a pipette. In the first of these is placed, with a normal drop-pipette, 6 drops of the culture the number of living bacteria of which is to be determined. With a fresh drop-pipette 10 drops of this are taken and deposited on a small Petri dish, and 6 drops that are dropped into tube No. 2. This is shaken and with a new drop-pipette 10 drops are taken and 6 drops as from No. 1 and so on with all the 7 tubes. In each of the Petri dishes is poured 12 c. c. of bouillon agar that is kept fluid at a temperature of 40° to 45°. The culture and the agar are carefully mixed by rocking the dishes up and down. When the agar has congealed the dishes are placed in the thermostat. After a lapse of 15 to 30 minutes the dishes are opened, in order to evaporate the exuded condensed water, whereupon they are again closed and placed at a temperature of 37° for 38 hours. It is necessary to allow the condensed water to evaporate as the bacteria would otherwise multiply in it. In 38 hours the colonies on and in the agar are counted. The 7 tubes constitute a series of dilutions ($^1/_{10}$-$^1/_{100}$-$^1/_{1000}$ etc.) and from each tube is seeded $^1/_2$ c. c. (10 drops). From the number of colonies counted and the degree of dilution, the number of living bacteria is calculated per c. c.

During the experiment seedings were likewise taken from the flask itself at the intervals stated, in order to be able to compare these with the quantitative determinations of lysin and bacteria.

Experiment No. 1.

200 c.c. bouillon.

Lysin concentration therein $^1/_{5000000}$. Lysin: coli d'H.

5 normal drops 24 hours old bouillon culture. Coli d'H.

Degree of dilution:	$^1/_{10}$	$^1/_{100}$	$^1/_{1000}$	$^1/_{10000}$	$^1/_{100000}$	$^1/_{1000000}$	$^1/_{10000000}$	Bact. per c.c.
Sample taken after:								
0 hours		420	56	0	0			98000
$^1/_2$ hour		573	76	7	0			133000
1 -		679	105	12	0			172900
$2^1/_2$ hours		7	12	15	0			62000
3 -		5	2	0	0			7000
$3^1/_2$ -		0	0	0	0			0
4 -		0	0	0	0			0
$4^1/_2$ -		0	0	0	3			0
5 -		4	1	0	0			1400
$5^1/_2$ -		1	0	0	0			100
6 -		18	1	0	0			2800
17 -					1470	156	21	3600000

Degree of dilution:	$^1/_{10}$	$^1/_{100}$	$^1/_{1000}$	$^1/_{10000}$	$^1/_{100000}$	$^1/_{1000000}$	$^1/_{10000000}$	Plaque-producing units pr. loop
Sample taken after:								
0 hours	0	0	0	0	0	0	0	0
$^1/_2$ hour	0	0	0	0	0	0	0	0
1 -	18	2	0	0	0	0	0	72000
$2^1/_2$ -	+	+	+	42	1			104000000
3 -	+	+	+	291	22	0	0	1022000000
$3^1/_2$ -	+	+	+	214	20	1	0	828000000
4 -	+	+	+	183	18	0	0	726000000
$4^1/_2$ -	+	+	+	253	8	0	0	664000000
5 -	+	+	+	303	15	+	1	906000000
$5^1/_2$ -	+	+	+	252	20	2	0	1104000000
6 -	+	+	+	187+	37	2	0	1140000000
17 -	+	+	+	310	20	4	0	1020000000

In the flask there was no macroscopically visible cloudiness during the first 6 hours of the experiment.

The direct seedings yielded the following result:

0 hours: 2—300 scattered colonies without signs of lysin.

$^1/_2$ hour: about 200 - - - - - -

1 hour: about the same number of colonies that on the one side of the plate have run together; here 20—30 plaques are found.

$2^1/_2$hours: Plate sterile.

3 - One colony.

$3^1/_2$ - Plate sterile.

4 - - -

$4^1/_2$ - One colony.

5 - 1 small, 2 large resistant colonies.

$5^1/_2$ - 2 - 3 - — —

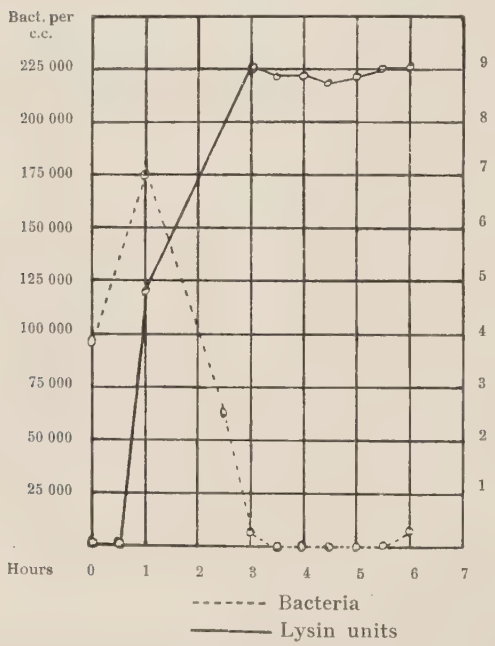
6 - 2 - 11 - — —

The experiment shows that the process, in all essentials has proceeded as in the earlier test-tube experiments.

In 6 hours with short intervals specimens have been taken, and thereupon after 17 hours.

The bacillar concentration was, to begin with, 98000 bacteria per c. c., this increased in one hour to 173000, thereupon in the course of 1 hour it fell to 0, remaining at that for one hour when it again rose.

Experiment I.



The concentration of plaque-producing units was at the commencement of the experiment less than 1, rose steadily during the whole experiment to a maximum of about 1000 millions per loop.

Reaction velocity: 3 hours.

Reaction maximum: 1000 million per loop.

On the curve, along the ordinate, the number of bacteria per c. c. is stated and the number of plaque-producing units per loop expressed by the negative exponent to 10.

It will be seen that the number of plaque-producing units at the commencement rises slightly with the number of bacteria and after one hour rises violently with a simultaneous fall in the bacterial figure to 0. In three hours the lysin concentration has reached its maximum.

By the direct seedings it will be observed that the course of the process in such respects is similar to that in the previously mentioned test-tube experiments.

Experiment No. 2.

200 c.c. bouillon.

Lysin concentration therein: $^1/_{5000}$. Lysin coli d'H.

5 normal drops 24 hours old bouillon culture. Coli d'H.

Degree of dilution:	0	$^1/_{10}$	$^1/_{100}$	$^1/_{1000}$	$^1/_{10000}$	$^1/_{100000}$	$^1/_{1000000}$	$^1/_{10000000}$	Bac. per c.c.
Sample taken after									
0 hour			1068	114					218200
1 -				225	20				425000
$1^1/_2$ hours				458	57	5			1028000
2 -				192					384000
3 -		0	0	0	0				0
$3^3/_4$ -	69	5	58	0					119
$4^1/_4$ -	205	30	3	0					505
5 -	586	65	6	1					1236
$5^1/_2$ -		194	29	0					4840
$6^1/_4$ -		801	86						16610
$6^3/_4$ -			258	18					438000
$8^3/_4$ -	Colonies have run together under the agar.								
$9^1/_4$ -	Innumerable.								
$9^3/_4$ -					985	91	8	1	18950000
$10^1/_4$ -						218	24	4	45800000
$10^3/_4$ -						463	43	5	89300000
$11^1/_4$ -						830	73	4	156000000
$11^3/_4$ -						1101	124	10	234400000
$12^1/_4$ -							144	14	284000000

Degree of dilution:	1/10	1/100	1/1000	1/10000	1/100000	1/1000000	1/10000000	Plaque-producing units per loop
Sample taken after:								
0 hours	+	+	+	+	52	8	0	
1 hour		lost						
1 1/2 hours	0	0	0	0	0	0	0	0
2 -	15	8	0	0	0	0	0	390000
3 -	+	+	+	175	57	1	0	149000000
3 3/4 -	+	+	+	562	21	7	0	154000000
4 1/4 -	+	+	+	+	74	6	0	2680000000
5 -	+	+	+	182	26	2	0	816000000
5 1/2 -	+	+	+	+	52	8	0	2640000000
6 1/4 -	+	+	+	+	66	6	0	2520000000
6 3/4 -	+	+	+	+	57	2	0	1540000000
8 3/4 -	+	+	+	+	84	2	0	2080000000
9 1/4 -	Ampule leaky, water has penetrated							?
9 3/4 -	+	+	+	+	66	2	0	1720000000
10 1/4 -	+	+	+	+	42	2	0	1240000000
10 3/4 -	+	+	+	+	48	4	0	1760000000
11 1/4 -	+	+	+	+	45	2	0	1300000000
11 3/4 -	+	+	+	+	74	3	0	2000000000
12 1/4 -	+	+	+	+	80	2	0	2000000000

The results of the direct seedings were as follows:

0 hours: Scattered and confluent growth of about 225 colonies, no signs of lysin.

1 hour: Same number of colonies, in the confluent areas plaques are observed.

1 1/2 hours: More plaques and very »nibbled« colonies.

2 - 10 very »nibbled« colonies.

3 - Plate sterile.

3 3/4 - 3 large resistant colonies.

4 1/4 - 3 - — —

5 - 3 - — —

5 1/2 - 9 - — —

6 1/4 - 40 - — —

6 3/4 - Scattered and confluent growth of large resistant colonies and small clear bluish ones in equal numbers.

8 3/4 - Confluent growth with numerous small plaques.

9 1/4 - — - - — - -

9 3/4 - — - - without plaques.

10 1/4 - — - - -

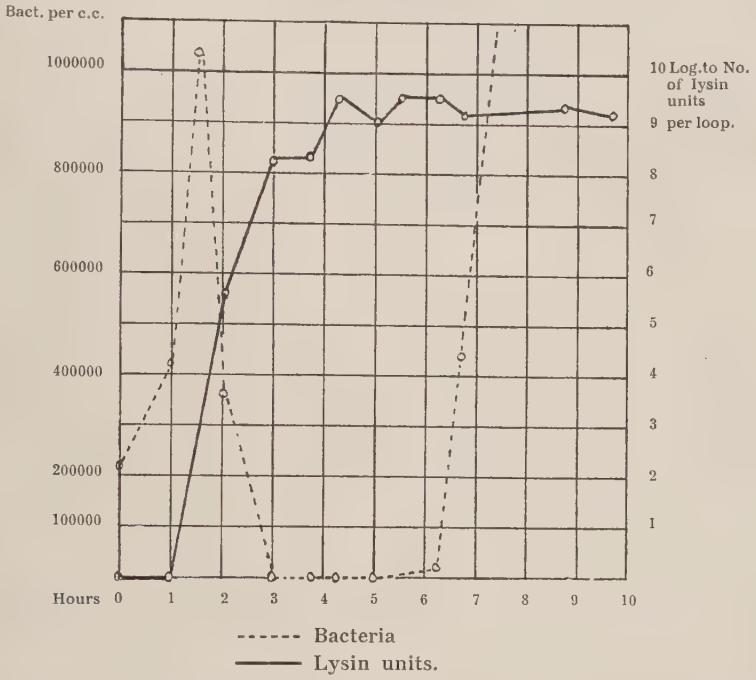
$10^3/_4$ hours: Confluent growth without plaques.

$11^1/_4$ - — - - -

$11^3/_4$ - — - - -

$12^1/_4$ - — - - -

Experiment II.



This experiment shews the same proportions as experiment No. 1. The duration of experiment is extended to 12 hours.

Whereas the lysin concentration here as in the first experiment has reached its maximum in the course of 4—5 hours, in this experiment we can follow the rise in the bacillar concentration to maximum; this was not the case in the first experiment.

The direct seedings demonstrate beautifully the same proportions as found in the test-tube experiment. A rising number of plaques is obtained until the plate is sterile and then an increasing number of colonies that finally run together and cover the plate. Thereupon again a small number of plaques that increases at every seeding.

Both these experiments shew, moreover, that to the fluctuating number of plaques, there is no corresponding fluctuation in the num-

ber of plaque-producing units, but that these increase steadily to maximum and remain at that stage.

The fact that the number of plaques fluctuates in the manner stated, must therefore be due to the changing susceptibility to the lytic action of the bacteria present or that the new generations that grow out possess a greater susceptibility than the preceding ones.

Experiment No. 3.

was carried out to discover whether the maximum values of the bacterial concentration and the concentration of the plaque-producing units were maintained during an experimental period exceeding 12 hours. The experiment proves that such is the case.

200 c.c. bouillon. Lysin concentration therein $^1/_{5000}$.

5 drops of culture 24 hours old. Coli d'H.

Samples were taken as follows, one immediately and thereupon at the times stated from the 10th to the 20th hour.

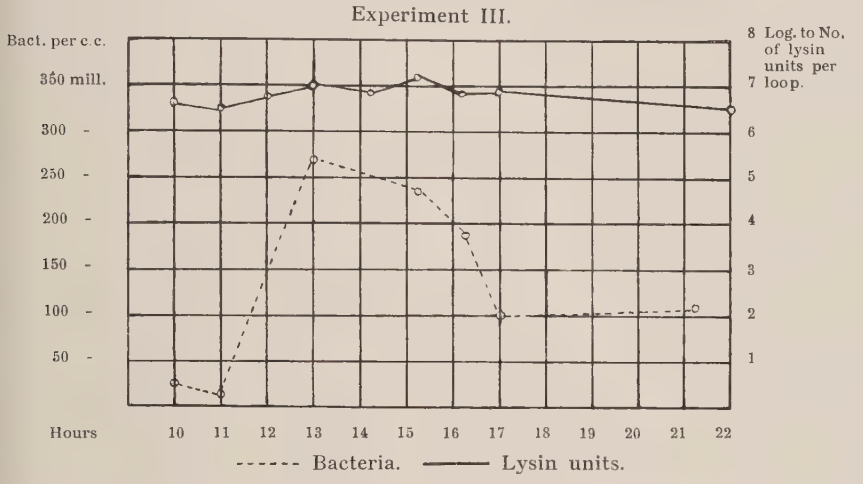
Degree of dilution:	$^1/_{10}$	$^1/_{100}$	$^1/_{1000}$	$^1/_{10000}$	$^1/_{100000}$	$^1/_{1000000}$	$^1/_{10000000}$	Bac. per c.c.
Samples taken after:								
0 hours	not counted							
10 -				1152	126	run together		24120000
11 -				+	+	10	0	20000000
12 -				?	?	?	?	?
13 -				+	+	106	17	270000000
$14^1/_4$ -				0	0	0	0	0
$15^1/_4$ -				+	+	35	10	235000000
$16^1/_4$ -				+	+	94	10	194000000
17 -				+	99	10	0	100000000
$21^1/_2$ -				+	+	69	4	109000000

Degree of dilution:	$^1/_{10}$	$^1/_{100}$	$^1/_{1000}$	$^1/_{10000}$	$^1/_{100000}$	$^1/_{1000000}$	$^1/_{10000000}$	Plaque producing units per loop
Samples taken after:								
0 hours	0	0	0	0	0	0	0	0
10 -	+	+	+	+	45	1	0	5500000
11 -	+	+	+	+	34	1	0	4400000
12 -	+	+	+	+	61	1	0	7100000
13 -	+	+	+	+	62	4	0	10200000
$14^1/_4$ -	+	+	+	+	44	4	0	8400000
$15^1/_4$ -	+	+	+	+	85	5	0	13500000
$16^1/_4$ -	+	+	+	+	58	2	0	7800000
17 -	+	+	+	+	37	4	0	7700000
$21^1/_2$ -	+	+	+	+	32	0	0	3200000

From the direct seedings no sign of lysin was seen.

In the same manner the 2 other coli strains were examined viz. coli Lantz and coli Tietz. In respect to these the experiments took exactly the same course and therefore they will not be reported.

It was my intention also to investigate other bacterial groups, but at the time in question, pressing reasons obliged me to proceed with other researches and later there has been no opportunity of continuing these experiments.



Some individual experiments, however, demonstrated that the conditions are probably the same in respect to dysentery bacilli. That this is also the case with all other bacteria with corresponding lysins, is evident from the fact that numerous non-quantitatively made experiments with these, progressed in exactly the same manner as the quantitatively investigated.

One problem, however, that was not solved by these experiments was the question as to the evaluation of the potency of the lysins, on the basis of the three conceptions set up.

Reaction figure,
Reaction velocity and
Reaction maximum.

There certainly exists the possibility that the two latter are identical in all lysins. Should this be the case, one can as do the majority of authors, suffice by determining the number of plaque-producing units. Proof, however, lacks that this procedure is correct.

Should it prove to be the case, it is at the same time proved that all lysins are actually of the same potency or by cultivation in series can be raised to it.

This, to a great extent, would tell in favour of the theory that there exists only one lysin that merely appears under varying forms.

The experiments show simultaneously that the lysin concentration does not arrive at maximum before the bacillary concentration commences to fall; this result that agrees with that of Otto and Munter proves that the rise in lysin may be due to the decay of the bacteria.

THE FORMS OF MANIFESTATION OF THE LYSIN.

How does the lysin manifest itself?

The form under which the lysin originally was recognized was as bare areas on a surface culture and this form is the most recognizable.

The plaques may be large or small, from scarcely visible to 8—9 mm in diameter. The culture may be entirely dissolved in the plaques, so that one can see nothing but the bare agar at the bottom of plaque, or it may be partially dissolved so that at the bottom of it, a thick or thin layer of bacteria may be observed.

The plaques are, as a rule, circular, with sharply defined margins. They may be surrounded by a zone, in which the bacteria are not dissolved, but where the culture is more transparent than in the area unaffected by the lysin. Should the plaques collide, they may run together forming large areas where no bacteria grow. The plaque-producing units in a fluid culture may be so numerous that by seeding on solid substrata no growth is obtained. At the border of the area upon which is seeded one will, as a rule, find a rim of culture; this rim towards the bare area of the plate has a lingular contour that corresponds to the confluent plaques, whereas the contour out towards the unseeded portion of the plate is even.

It would be expedient to distinguish between the manner in which the lysin manifests itself in fluid culture, and that when seeded on solid substrata. In both cases one must distinguish between things

observed macroscopically and those that can only be seen microscopically.

In fluid substratum the lysin manifests itself, either by wholly or partially inhibiting the growth for a long or short period. Frequently when a sufficiently long period has elapsed growth of bacteria will be obtained, and this manifests itself, as a rule, as a precipitation, consisting of resistant bacteria. A total inhibition of the growth I, personally, have never observed, but it has been described by several authors. This applies only when the substratum has been seeded with a small quantity of culture. If large quantities of culture have been seeded, so that a cloudiness has appeared in the substratum, every visible sign of the presence of the lysin may fail to appear, or one observes the cloudiness decrease somewhat only to increase later.

If a small quantity of lysin and bacteria is added to a fluid substratum, incubated at 37^0 and seeded at short intervals on solid substratum, the results previously mentioned are obtained.

The seedings shew an increasing number of plaques that finally run together forming bare areas. Thereupon an increasing number of resistant colonies appear. These latter will be made the subject of special mention, presenting as they do very interesting circumstances, and also giving a good deal of information as to how the plaques may be presumed to originate.

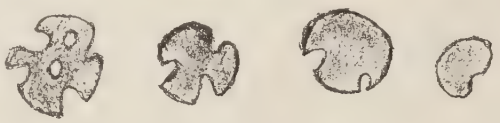
Among the resistant colonies there are two forms that are easily distinguishable. The one form manifests itself as large, whitish, very saturated, at times almost mother-of-pearl-like colonies, the other as small transparent colonies with a bluish tint.

The ratio between the number of these colonies is in definite relation to, how long the process has progressed. To begin with one will find a considerable number of the small bluish colonies and none or very few of the large saturated ones. Then the small ones decrease in numbers and finally disappear entirely, whereas the large ones increase in numbers until they, at last, cover the whole plate. The plate is now covered with a greasy, saturated culture, but during the following spreadings it will be seen that this aspect changes, becoming gradually less saturated and finally quite resembling a normal culture. I a certain number of cases, the development continues in this manner, that in this culture some few plaques are obtained that later become more numerous.

Besides this changing relationship between the two sorts of colonies, it is interesting to note their mutual geographical relations.

The large saturated colonies are frequently very »nibbled« at the margins, others, besides this, are perforated with several holes. This may even happen to such a degree, that one finds nothing but small remains of a colony, the »holes« having eaten away the majority of it (Drawing I).

I.



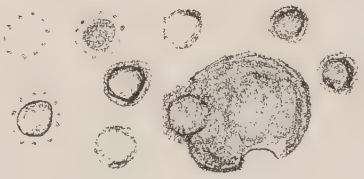
Should the number of small bluish colonies be great, it will, as a rule, be found that many of the existing large colonies are »nibbled« in this manner. This is, however, never the case when there are none, or only few of the small bluish colonies.

The explanation of this circumstance now proves to be, that it is these small bluish colonies or their invisible remains that produce the notches or perforations in the large ones. In the first place we frequently observe a small colony occupy a notch in the large ones, but even where this is not the case it must be presumed that, either one has been there and has subsequently dissolved, or there must have been one, in an embryo state, that never developed, but dissolved. This is made probable by the following observation: If an agar plate containing numerous saturated, »nibbled« and small bluish colonies is allowed to stand at room-temperature, around the small bluish colonies a reef of a more lustreless culture grows out. This is the case both with the isolated colonies as with those lying in the notches of the large ones. But this is not all. On the bare areas of the plate, where apparently there are no colonies, or there have never been any, these reefs grow out so that one sees a spot the size of the ordinary lysin plaques surrounded by a reef, that in appearance resembles closely the reef around the small bluish colonies. The reef is not always continuous, but may consist of isolated colonies. Such circles of small isolated colonies may also be observed isolated on the plate (Drawing II).

If we now turn to the conditions in the fluid substratum of which the seedings mentioned here are a reflexion, it is my opinion that these are to be viewed in the following manner:

To begin with, there are a great number of bacteria that are susceptible to lytic action and a very small number of the insusceptible. The susceptible form small bluish colonies, the insusceptible large saturated ones. By dissolution of the small ones, plaques are formed on the plate and in the fluid substratum the lysin increases in quantity. At a certain juncture there are so few bacteria present that by spreading, one does not obtain a confluent growth, but scattered colonies only. Some of these consist of susceptible (small bluish), others of insusceptible bacteria (large saturated). The colonies consisting of the susceptible, steadily decrease in numbers, whereas the resistant increase until they finally cover the plate. What happens then with the resistant cannot with certainty be decided, but it is probable that they gradually change character and become susceptible. If this is not the case, it must be presumed that new batches develop that are susceptible.

II.



THE MICROSCOPIC EXAMINATION.

The object of this was to discover:

I. The origin of the plaques in the susceptible culture.
Do the bacteria first grow out and are thereupon dissolved, or are they dissolved immediately?

II. Are the resistant bacteria already present in the culture or are they later generations of the susceptible bacteria?

III. How does the dissolution of the bacteria occur?

The experiments are so arranged that a mixture of lysin and bacteria is seeded on an agar plate, placed at 37° and at short intervals examined microscopically.

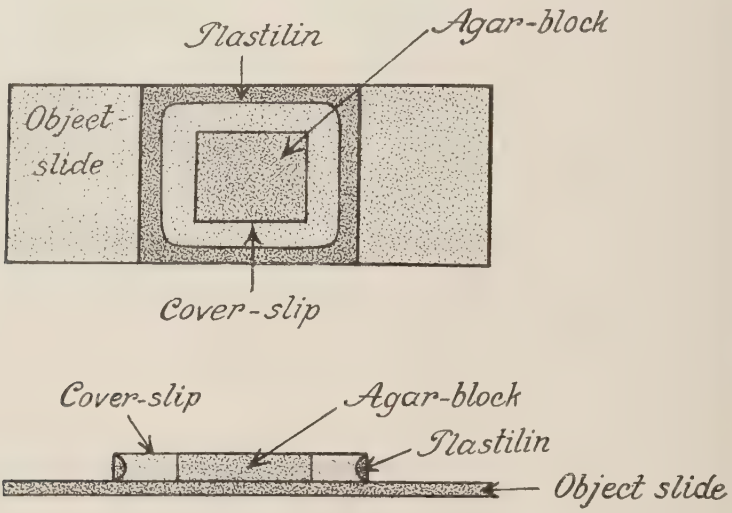
The first question that presented itself was what quantity of bacteria it was most practicable so seed.

The quantity of bacteria was selected in such a manner that the bacteria, when two drops were spread on an agar plate, lay so closely that several bacteria were visible within any visual field.

On account of the quantity of lysin two parallel experiments were invariably undertaken, and in such a manner that in the one the volume of lysin was enough to ensure obtaining so many plaques that they ran together forming bare areas, and in the other the quantity of lysin was such that isolated plaques resulted.

The seeding of the two drops was undertaken with a thin goose-necked glass rod.

III.



The agar plates used had to be fairly thin for the sake of transparency.

The examination of the agar plates might be made in two ways.

I. A square piece is cut out of the agar plate with a sterile knife, placed on a large object slide and covered with a cover-slip. The cover-slip must be so large that it, at all four corners, overlaps by some few millimeters the agar block.

The four sides of the cover-slip are now affixed to the object glass by means of plastilin and a warm knife, in such a manner that the plastilin is nowhere in contact with the agar block; in this manner a moist chamber is obtained (Drawing III). The preparation is placed on an object-board, an immersion lens is adjusted and the microscope is placed in a heated box. Hereby it is possible to observe the development continually for several hours.

I. The agar plates are placed in a thermostat and at short intervals pieces are cut from them. These are placed on object glasses and examined first with a dry lens and then with an immersion lens. The examination can be made immediately the pieces have been cut out, or they can be treated with formaldehyde by placing them in a Petri dish together with a few drops of concentrated formaldehyde solution on a piece of filter paper. Thereupon all preparations can be examined at once which faciliates the general survey.

For the first experiment the former method was employed because it was desirable to observe the development continually.

This method is, however, rather more complicated than the other and, moreover, possesses the disadvantage that when working with bacteria that in fermenting general air, air bubbles formed under the cover-slip preventing further observation. As it proved that by the second method equally favourable results were obtained and after it, during many experiments, had been included as a control, I proceeded to employ it exclusively.

EXPERIMENTS.

Lysin: Lantz.

Bacteria: Coli Lantz.

In the concentration $^1/_{10}$ this lysin yielded a completely clear plate covered with resistant bacteria only. At the edge out towards the unseeded part of the plate a reef was as usual found that inwards towards the centre of the plate had a lingular contour and outwards was quite even.

The microscopic picture of the course of the process is shewn by the drawings.

The plate was seeded at 4 p. m. On the 4 o'clock preparation we observe the bacteria lying fairly closely and unaffected by the lysin. At 4^{30} with a strong dry lens (ca. 750 mag.) some few sharply defined bacteria are seen that subsequently prove to be resistant. They are surrounded by some shadowy, irregular figures that are the partly dissolved bacterial colonies. If we observe a preparation from the same moment with an immersion lens (mag. 1000) we note the sharply defined resistant bacteria and among these the non-resistant

about to be dissolved. They manifest themselves as large circular oval or slightly irregular faintly refractive bodies, supplied with one or several refractive granules.

5 p. m., half an hour later, the circular bodies have become still less refractive, whilst the resistant bacteria are found in incipient division.

The development now continues by the circular bodies becoming more and more indistinct, whilst the resistant bacteria gradually grow into small colonies, as will be seen on the drawings from 6^{30} and 7. On the latter we see that the resistant colony is close to some bacteria that are not yet quite dissolved, and furthermore we note that the resistant bacteria have become very large, and are provided with irregular zones.

In the concentration $^{1}/_{100000}$, lysin Lantz yielded numerous isolated plaques. The microscopic illustration of the formation of these is shewn on the drawings.

The plate was seeded at 4 p. m.

On the preparation from 4 o'clock we see the bacteria unaffected by the lysin. At 4^{30} (not illustrated) we note that the bacteria have become somewhat larger, but division has not yet set in.

At 5 o'clock, the drawing shews the spot where later a plaque appears. We observe that the bacteria have divided and form minor colonies consisting of several individuals. A couple of these are very faintly refractive, they have commenced to dissolve.

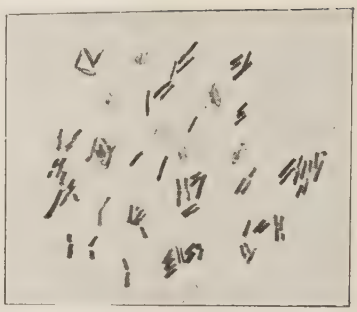
5^{30}. We observe that the colonies have become somewhat larger and that dissolution is in full swing. Moreover we remark that the dissolution takes place from that side of the plate facing the centre of the hole, whereas that part of the colonies facing in the opposite direction consists of sharply defined individuals. One of the bacteria in the dissolved colonies, however, has retained its clear contours. These are the resistant bacteria. This circumstance is noteworthy as it makes it probable that the culture, from the commencement, has consisted of two kinds of individuals, resistant and susceptible.

6^{00} and 6^{30} p. m. the same is observed, but is more pronounced, and the hole now begins to assume its outer boundaries, the colonies here having become so large that they run into each other. The resistant at the same time have become somewhat longer.

COLI LANZ

DILUTION OF LYSIN $^{1}/_{100000}$.

1. 4.00 p. m. before the commencement of the experiment. Strong dry lense (mag. c. 750).

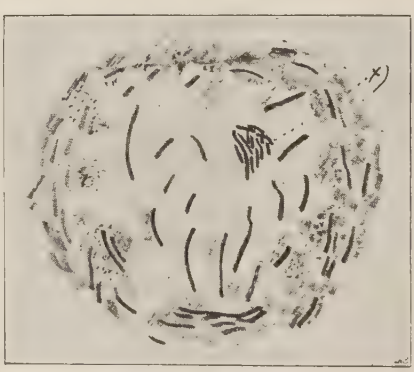


2. 5.00 p. m. Strong dry lense (mag. c. 750).

3. 5.30 p. m. Strong dry lense (mag. c. 750).

4. 6.00 p. m. Strong dry lense (mag. c. 750).

5. 6.30 p. m. Strong dry lense (mag. c. 750).



6. 7.00 p. m. Strong dry lense (mag. c. 750).

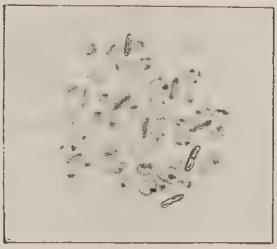
*) This colony of resistant bacteria was drawn from the preparation at 8.45 p. m.

COLI LANZ

DILUTION OF LYSIN $^1/_{10}$.

1. 4.00 p. m. before the commencement of the experiment (strong dry lense, mag. c. 750).

2. 4.30 p. m. (mag. c. 750).



3. 4.30 p. m. Immession lense (mag. c. 1000).

4. 5.00 p. m. Immession lense (mag. c. 1000) (6.00 p. m. = 5.00 p. m.).

5. 6.30 p. m. Immession lense (mag. c. 1000).

6. 7.00 p. m. (Immession lense mag. c. 1000).

7. Resistant bacterium (Scheme).

7^{00} p. m. The hole is now essentially fully formed and the resistant bacteria have grown into long bodies and some of them have commenced division.

8^{45} p. m. The picture is, in the main, the same, except that the resistant bacteria now form minor colonies.

The experiment shews, that the result differs all according to whether a lysin concentration of $^1/_{10}$ or $^1/_{100000}$ is used.

If we use a concentration $^1/_{10}$ we observe the bacteria within the field of vision, gradually dissolve with the exception of some few that remain lying and later develop into colonies. The dissolution of the individual bacteria takes place by the bacteria swelling up considerably, losing their elongated form and becoming circular bodies that gradually lose their refractive powers, becoming dull in outline. Simultaneously they get one or two refractive granules that are observed to lie near the extreme margin of the circular body. Should these bodies lie in such a manner that they collide, they do not retain their circular form, but become angular. In the course of 5-6 hours they, as a rule, disappear entirely, without it being possible to see how this final stage of the dissolution takes place.

The resistant bacteria are already, at this juncture, distinguishable from the susceptible by the fact, that in contrast to these, they assume sharper outlines, become elongated and develop zones (see drawing). In the course of 3 or 4 hours they divide and develop gradually into colonies.

If, on the contrary, a concentration $^1/_{100000}$ is used, we observe the bacteria develop into colonies of up to 10 individuals, that not until then commence to dissolve. This dissolution, that, as regards the isolated individuals occurs in the same manner as when concentrated lysin is used, appears in patches. Within a definite circle, we first find some few, later several minor colonies that are in incipient dissolution. The circle is bordered by isolated colonies that shew no signs of dissolution or only inwards towards the centre of the circle where they are in incipient dissolution. The circle becomes more and more confined, the colonies that border it running together and the colonies in the centre dissolving more and more. The resistant bacteria only, are now seen sharply outlined and in multiplication.

A problem of great interest, viz. why the lysis does not go on from colony to colony over the whole plate was not solved by this experiment.

The transition from plaques to the natural portions of the culture are not defined, swollen, natural and resistant bacteria being found intermixed on this spot. Within the area of the plaque we frequently observe that the lysin, from a colony that is in incipient dissolution, attacks another in which hitherto no lysis has been noted, the unaffected colony becoming so large that it gets into contact with that about to be dissolved. The fact that the phenomenon is limited to the circular area must be due to unknown reasons. The elucidation of these would be of great significance, for the limited action of the lysin is one of the main arguments in favour of it consisting of individual living organisms that formed colonies viz. the plaques.

Besides the coli strain mentioned here, another coli strain, coli d'H. with corresponding lysin was examined. This yielded the same results, the lysis, not commencing until when the plate was covered with a single layer of bacteria.

A typhoid strain T3, with corresponding lysin shewed the same circumstances. The lysis began, in this case, when the single layered colonies were so large that they began to run into each other.

A staphylococcus strain Staph. d'H. with corresponding lysin shewed identical circumstances. The globular bacteria swelled up, as did the rod-shaped, into large circular, slightly refractive bodies, that, without one being able to follow the process in detail, gradually became invisible.

A paratyphoid strain — K. M. with corresponding lysin deviated, however, from the others. At an early juncture we here note that when the bacteria had formed colonies of 2—3 individuals that some of these shewed signs of incipient dissolution. This, however, was not continued, but the bacteria that had shewn no signs of dissolution developed further and covered the whole plate. Not until then was incipient lysins again observed, the culture clearing in patches by the bacteria losing their contours and being left behind as an amorphous layer in the bottom of the hole. Later on in this mass there developed long filiform bacteria and these gave to the plaque a radially striped appearance.

If we return to the point of departure of these experiments the questions posed must be answered in the following manner.

The bacteria are dissolved by a concentrated lysin immediately (in the course of 3 hours), apparently without having multiplied. In a diluted lysin they develop into single layered colonies of varying size. In these colonies the lysis sets in on one single spot and then spreads over the whole culture. One colony that is in dissolution, may, when it gets into touch with another colony, that hitherto has shewn no signs of dissolution, transplant the lysis to this.

The resistant bacteria are already present in the otherwise susceptible culture.

The dissolution of the individual bacteria occurs in the manner described, by their swelling up considerably and simultaneously gradually losing their contours.

The plaques are formed in the manner described, by minor colonies, within a circle, dissolving whilst simultaneously similar colonies outside the circle develop collide and thereby limit the plaques.

PREPARATION OF LYSINS.

The lysins are, as already mentioned, demonstrated in nature in many different places and in many different media. From their occurrence it has not been possible to come to any conclusion regarding their nature, whether it ts a question of a virus or a ferment.

One would get an indication in that direction if it were possible, at will, to produce lysins that were capable of dissolving any bacteria chosen. This has also been attempted numerous times, sometimes with apparent success. The experiments have, however, all had the same fault, viz. that it was impossible absolutely to exclude the lysins being present in the various things used in their production. This thus applies to experiments on the production by injection of bacteria into the peritoneum of experimental animals, and by the filtration of old cultures. Both these methods have also only been successful in individual cases, and this denotes, more than anything else, that it was not a question of production, but only of a demonstration of lysins already present.

The reason why I entered upon experiments on the production

of lysins was, that I was faced with the exigency of treating both bacilli carriers and patients with chronic infectious diseases in the intestines or urinary ducts. Here it was essential to produce quickly and in the simplest possible manner, lysins that could dissolve the bacteria presumed to be the cause of the disease.

To begin with, intra peritoneal injections into guinea pigs of the bacteria one desired to dissolve, were attempted. In spite of numerous experiments success did not crown these endeavours to demonstrate lysin in the exudates produced. The bacteria experimented with were, a coli strain, a paradysentery strain and a paratyphoid strain. The bacteria were injected in the first experiments living, in rising doses at intervals of one or several days. As it proved that the experimental animals could only stand very small doses administered in this manner, I tried giving one or two injections of killed bacteria at intervals of two days, and the animals were then enabled to withstand far better injections of living bacteria. The result, as already mentioned, was invariably negative. The explanation of the fact, that in some cases it may be successful, but in the majority unsuccessful, is probably as follows: If we inject bacteria into the peritoneum of a guinea pig and the animal simultaneously has a lysin in its intestines that is capable of dissolving the bacteria, the lysin passes by way of the blood vessels or lymph ducts to the peritoneum and dissolves the bacteria injected, whereupon it increases greatly and is demonstrable in the exudate. In all cases, where, in the experimental animal used no lysin was present, capable of dissolving the bacteria, the experiment was unsuccessful. Whether this explanation is correct would be decided by injecting bacteria, against which we possess a lysin, and simultaneously administering the lysin to the animal per os.

As the object of these experiments was to produce lysin against such bacteria against which we have no lysin, no experiments of the aforementioned description were undertaken.

Simultaneously experiments were made to see whether bacteria by being cultivated in bouillon four weeks without re-seeding should themselves produce lysin. All experiments in this direction gave negative results. Consequently we had to limit ourselves to the treatment

of such patients whose bacteria were dissoluble by the lysins we had at our disposal.

At a later juncture Borchardt's work on the occurrence of lysins in the alimentary canal of animals appeared. In this work it was stated that lysins could be produced in the following manner:

A cat is starved for 24 hours whereupon it is killed. Duodenum and pancreas are removed. Duodenum is opened and the mucous membrane is carefully rinsed with physiological saline solution, whereupon the scraped mucous membrane is pounded in a morter and mixed with physiological saline solution (50 c. c. per 10 gr. organ), then it is filtered, first through a paper funnel and afterwards through a Berkefeld filter. The removed pancreas is treated in the same manner. The pancreas and duodenal extracts are mixed in the proportion 2 to 3 and from the mixture 3 c. c. are added to 8 c.c. bacterial culture. It is incubated at 37° and seeded at short intervals on agar. In the seedings lysin plaques should be found.

By this technique several experiments were carried out and the research of lysin was continued by repeated filtratrations of the culture with the accompanying examination of the filtrates.

The bacteria tested in this way were the following:

Dysenteriae Shiga V. J.	Typhoid T3.
— Kruse V. J.	Paratyphoid P.
Coli C. B.	— 827
- U. I.	Paradysenteriae Flexner
- P.	— Strong
Staph. Cereus alb.	and Coli Lantz
- aureus	

Several of the bacteria used could be dissolved by the lysin found in the lysin collection. The possibility that, the reason for the negative results ought to be sought in the bacteria, was therefore excluded, and it had therefore to be sought in the technique.

From the idea that the lytic action in many respects reminds one of an autolysis, and that possibly one ought to arrive at a better result if the organs used were allowed to enter upon incipient autolysis, the following technique was experimented:

The organs were removed immediately the cat was killed and were (duodem unopened) left untouched for 24 hours af room-tem-

Experiment I.

	Seeding immediately	After 1¾ hours	After 4 hours		Seeding immediately	After 2¼ hours	After 4¼ hours		Seeding immediately	After 1½ hour	After 3 hours
Paratyphoid 1022	—	—	—	After 24 hours heated for ½ hour to 54° and 1c.c. transplanted to fresh cultures.	—	—	—	After incubating 24 hours at 37° heated to 54° and 1c.c. transplanted to fresh cultures.	—	—	—
Coli Lantz	+	+	+		+	+	+		+	+	+
Dysenteriae Shiga	—	—	+		—	—	—		—	—	—
— Kruse	—	—	+		—	—	—		—	—	—
Typhoid T3	—	—	+		—	—	—		—	—	—
Staph. cer. alb.	—	—	+		—	—	—		—	—	—
Paradys. Flexner	—	—	+		—	—	—		—	—	—
— Strong	—	—	+		—	—	—		—	—	—
Coli Tietz	—	—	+		—	—	—		—	—	—

perature. The duodenal mucous membrane and the parenchyma of the pancreas (after the removal of the connective tissue) were pounded in a morter to the consistency of thin porridge. This was divided into 10 equally large portions that were placed each in its test-tube and stirred with 5 c. c. of sterile bouillon. From 24 hour cultures of the bacteria one desires to dissolve, a considerable quantity is suspended in each of the 10 test-tubes, in such a manner that the bacteria are well blended with the extract from the organs. After having stood in a thermostat for 24 hours at 37° they are inactivated in a waterbath at 54° for ½ an hour. A precipitation has now formed with a supernatant fluid. 1-2 c. c. of this is mixed with 5 c. c. of a sterilely prepared suspension in bouillon from a 24 hours old agar culture of the bacteria to be dissolved. The mixture is placed at 37° and is immediately seeded on agar at intervals of one to two hours.

As will be seen this technique deviates in the following from that of Borchardt.

The organs have lain for 24 hours at room-temperature before being used. The filtration

through paper and Berkefeld's filter is omitted. The organs are brought into direct contact with the bacteria without being diluted.

The results of a couple of experiments are given on the table overleaf.

In this experiment I was successful in producing lysin against one of the bacteria employed viz. Coli Lantz.

Experiment II.

	Seeding immediately	After 2 hours	After $3^1/_2$ hours	
Paratyphoid 827	—	gran.	—	
— 830	—	—	—	
Dys. Shiga V. J.....	—	++	+++++	Fairly deep holes.
- Kruse	—	—	—	
Staph. citr.	++++	++++	++++	Only resistant colonies.
- pyog. haem.	++	+++	+++	Numerous very small and superficial plaques.
Coli C. B.	gran.	gran.	gran.	
- Lantz.....	gran.	gran.	—	
- Tietz	—	—	—	
- X	+	+++	+++	Large but quite superficial plaques.
- 41	gran.	gran.	gran.	
- 42	gran.	gran.	gran.	
Paratyphoid 1022	gran.	gran.	gran.	
Paradys. Strong	gran.	gran.	+++	Deep, defined, limpid plaques.
Flexner.....	++	+++	+++++	Large deep, limpid plaques with remains of colonies in each.
- III.....	—	++	gran.	Large, quite superficial plaques, that distinctly emerge into granulated portions where they run into each other.
- II.	—	—	—	
- I.	—	—	—	
- Shiga Doerr...	—	+	+++	Large, fairly deep plaques.

On the table the following designations denote:

+ = plaques.

Several + = an increasing number of plaques.

Pl. clean. = the whole culture has been dissolved with or without the occurrence of resistant colonies.

Gran. = that the culture has had the previously mentioned granulated appearance.

After 24 hours the tubes are heated for $^1/_2$ an hour to 54° and 1 c. c. of the contents is added to a tube with fresh culture. The tubes are placed in a thermostat and seeding is carried out at the times stated.

Experiment II. (continued).

	Seeding immediately	After $1^1/_2$ hours.	After 3 hours.
Paratyphoid 827.....	gran.	gran.	gran.
— 830.....	-	—	-
— 1022.....	-	gran.	-
Coli C. B.....	-	-	-
- Lantz.....	—	—	—
- Tietz.....	gran.	gran.	—
- X.....	+++	+++	gran.
- 41.....	gran.	gran.	-
- 42..... ..	-	+++	+++
Staph. citr.....	-	++++	+++++
- pyogen. haem.	-	gran.	gran.
Dys. Shiga V. J.....	-	-	++
- Kruse V. J.....	-	-	gran.
- Shiga Doerr	—	++	-
Paradys. Strong	+++	+++	+++++
— Flexner	++++	++++	++++
— III.....	gran.	gran.	gran.
— II.....	—	—	—
— I.....	—	—	—

After 24 hours all tubes heated $^1/_2$ an hour at 54° and 1 c. c. of the contents added to new tubes with fresh culture. The tubes placed in a thermostat and seeding carried out at the times stated (see the next page).

The granulatet appearance may, as will be seen from the seedings from paradysentery III occur by very superficial plaques running into each other, consequently when there is a lysin present. This can, therefore generally be demonstrated by diluting the fluid by adding to it a frehs culture and seeding. By this means the isolated plaques are brought out. The granulated appearance can, however, also at

Experiment II. (continued).

	Seeding immediately	After 2 hours	After $3^3/_4$ hours	Final result
Paratypoid 827.....	—	—	—	gran.
— 830.....	—	—	—	-
— 1022.....	—	gran.	gran.	-
Coli C. B.....	gran.	-	-	-
- Lanz.....	—	—	—	-
- Tietz.....	—	—	—	-
- X.....	++	+++	++++	+
- 41.....	gran.	++	+++	+
- 42.....	-	gran.	gran.	+
Staph. citr.....	++	++	++++	+
- pyogen. haem.	—	—	—	+
Dys. Shiga V. J.....	gran.	?	+++	+
- Kruse V. J.....	-	gran.	—	gran.
- Doerr.....	-	-	++	+
Paradys. Strong	-	++	+++	+
— Flexner	+++	++++	++++	+
— III.....	—	—	—	+
— II.....				—
— I.....				—

times be observed in cultures that have never been in contact with lysin and in which it has not been at all possible to demonstrate any lysin Moreover I have observed the granulated appearance in the culture of the previously mentioned coli strain C. B. that originally was influenced by the lysin, but later was insensible to its action. Simultaneously with the change the surface of the agar culture that previously was ewen and smooth became cloudy and granulated.

The granulated appearance therefore cannot alone be regarded as a sure sign of the presence of lysin. Consequently in these experiments only distinct plaques are taken as proof of its prensence.

All the bacterial strains in the experiments were such, that numerous times had been re-seeded and spread without plaques having appeared in them. With the exception of paradysentery I., II. and III. all strains at earlier experiments were capable of dissolution by lysins.

In this experiment I was successful in producing lysins capable of dissolving 10 out of 19 bacterial strains.

The strains that in those experiments could be dissolved were the following:

2 dysentery strains
3 coli strains
3 paradysentery strains
1 staphylococcus citreus
1 — pyogenes haemolyticus.

Consequently by means of this technique success has crowned attempts to produce lysins that dissolve several different bacterial species.

What theoretical significance can be ascribed to this?

The assumption from which the technique was changed, to wit that it was a question of an autolytic process that originated in the autolysis of the organs used and thereupon was continued in the transmissible autolysis of the bacteria, seems to be the nearest explanation of the phenomenon, transmissible lysis.

If this is right, the theory of the invisible virus must be rejected, and it must be presumed that it is a question of an autolysis that, when once set in motion may be continued indefinitely, a theory that essentially agrees with that advanced by Bordet.

Another possibility is that the bacteria in the organs employed have, whilst lying for 24 hours, decomposed and that this has been transmitted the strains examined.

THE OCCURRENCE OF LYSIN IN TYPHOID AND PARATYPHOID PATIENTS.

Researches of this description were, a priori, of great significance.

In addition to discovering in how many cases lysins were demonstrable in these patients, it was important to get to know at what juncture in the disease these lysins made an appearance. d'Herelle already in his earliest publications states, that the occurrence of lysins invariably coincides with an improvement in the condition of the patient or with the final recovery. Should the patient be at a stage when the disease is increasing or an exacerbation sets in, lysins can never be demonstrated. He is, therefore, of the opinion that the

lysins may be used for producing improvement or recovery and this he, furthermore supports by his researches on avian typhosis.

As paratyphoid and more especially typhoid are diseases with a fairly characteristic course, it might, a priori, be presumed possible by researches in these patients to decide whether this contention was correct. If lysins were found to be present from the commencement of the disease, and the disease, in spite of this, retained its usual protracted course, not only the assumption that the lysin occurred simultaneously with the setting in of an improvement and to a certain extent was the cause of it, but also the possibility of conveying an improvement to the patient by means of lysin, should be rejected. Finally one might imagine the possibility of deciding, by means of the demonstration of a definite lysin in the feces, whether a patient suffered from one disease or the other. This would be of especial interest in cases where no bacteria were found in the feces. Such diagnostic application of the lysin is, however, placed out of the question by the demonstration that the lysins are, in a high degree, unspecific.

As material for investigations of this description, one would, a priori, regard a well-confined epidemic with well-ascertained source of infection as suitable. The reason why an epidemic of this description was to be preferred was the following. In a certain number of patients one had to reckon with the possibility of not finding bacteria, but if it were known that the source of infection was the same in all cases, one could also reckon with the bacteria being identical and consequently examine the lysins from specimens of feces from those patients in whom no bacteria were found, against the bacteria from other patients.

Instead of examining a number of scattered cases I, therefore, preferred to examine 2 epidemics that both fulfilled the aforementioned requirements.

The examination was carried out by examining, in the usual manner, all specimens of feces in search of pathogenic bacteria, and a mixture of these was used in the lysin test, both for the specimens of feces in which they were demonstrated, as also for those where no bacteria were found.

Both the epidemics investigated were »milk epidemics« their ori-

gin being traced in each case to a particular dairy. The one was a paratyphoid epidemic at Lemvig in Jutland, the other a typhoid epidemic at Bogense on Funen. All the patients were treated at the hospital and from there samples of feces and urine were sent for examination.

All the feces and urine specimens were, as already mentioned, examined for pathogenic bacteria.

The examination was carried out in the following manner: A lump of feces, the size of a pea was suspended in 15—20 c. c. sterile bouillon in a test-tube. From the urine specimens some few cubic centimeters were likewise mixed in a test-tube with 15—20 c. c. sterile bouillon. To these were added some drops of a culture of the bacteria one sought to dissolve. The reason for this was that one had to reckon with the fact that in a certain number of feces and urine specimens, no typhoid or paratyphoid bacteria were demonstrable. If lysin were in such a specimen, by suspension one would only attain at diluting this, there being no bacteria present at the expense of which an increase in the lysin could occur. Certainly the contingent lysin, in a number of cases was capable of dissolving, besides typhoid and paratyphoid bacilli, also coli bacilli and could thus be increased by the dissolution of these. As this circumstance, however, eluded, every control, I preferred to add the amount of culture mentioned.

The tubes were then placed in a thermostat a 37^0 until the next day and the contents were filtered first through paper and subsequently through a Berkefeld filter.

The Berkefeld filters used for several researches were cleansed by boiling in an enamel vessel for 10 minutes, rinsed carefully in ordinary water and again by passing a considerable quantity of destilled water through it. If this ran through slowly, the filter was partially blocked. In such a case it was boiled in ca. $^1/_{10}$ normal NaOH and again destilled water is passed through it. To ensure this being sufficient to remove all traces of NaOH the rinsing water was tested with phenolthalein. If it did not react to this, its reaction lay below a p_H figure of 8 which proved that the filter was sufficiently clean.

Thereupon the filtrates were examined for lysin.

As already mentioned a mixture of the bacteria isolated from the

feces specimens was used. This mixture was prepared in the following manner:

The bacteria to be included in the mixture were seeded the evening before in a bouillon tube. The next day all the cultures were examined microscopically and if found to be pure cultures of mobile rods, equal volumes of all the cultures are mixed together. With a couple of drops of this mixture as many boullion tubes of 15 to 20 c. c. as required are seeded and when the bacteria in the course of some hours have developed into a visible cloudiness 1 to 2 c. c. of the filtrate is added.

Spreading on agar is immediately undertaken, and this is repeated after $1^1/_2$ and 3 hours and again after 24 hours. The plates upon which has been spread are placed in a thermostat at 37^0 until the next day, when they are examined for signs of lysin. These consist partly of plaques designated on the tables by one or several +, all according to the number, otherwise one sees the plate smooth with isolated resistant colonies destignated by r. pl. Finally the culture may have a granulated appearance. As mentioned before this is not in all cases a sign of the presence of lysin and in the numerical account of the results, consequently, only those samples that yielded plaques or a clean plate are reckoned as positive.

As will be remembered plaques are only obtained when one seeds during certain phases of the process of dissolution. That we do not always hit on the right time in the three seedings undertaken, must be taken for granted, the added quantity of the filtrate containing greatly varying quantities of lysin. When the mixture of culture and lysin has stood in the thermostat at 37^0 for 24 or 48 hours, it is again filtered and the filtrate thus obtained is tested in the same way as the first. In a like manner a further filtration was undertaken, making a total of 3, followed by testing the filtrate. The three filtrates were designated: F. I., F. II. and F. III.

If this technique is carefully carried out, it yields, as will be seen by the following, good results.

One single mistake that caused a long series of actually positive specimens to turn out negatively, necessitating a repetition, will be related.

During the investigation of the typhoid epidemic, a paratyphoid strain was, by mistake, put among the cultures that were daily used

for producing the mixture for the lysin test. As paratyphoid bacill grow far more quickly then typhoid bacilli the cultures used arrive at consisting, for the most part, of paratyphoid bacilli. As all filtra tes originated from typhoid patients, the results all become negative until the mistake was discovered. There were, however, some ex ceptions, as some of the tests were positive. A closer examination of the filtrates from these specimens shewed that besides dissolving the typhoid strains they, in addition, dissolved the paratyphoid strain that, by mistake, had been put amongst them.

If we regard the purely technical results, we might ask why fil tration is carried out just 3 times.

We, a priori, had to set out with the assumption that the more often we filtered, the more positive results would be obtained. Three times was chosen as being most practicable.

What has been attained by filtering 3 times is shewn by the fol lowing comparison:

By filtering once 84 positive were found: 59% of all positive.
- — twice 113 — - - 79% - - -
- — 3 times 143 — - - 100% - - -

The reason why a larger number of positive are obtained at each filtration is, that the quantity of lysin per c. c. is increased every time the lysin is allowed to act upon a new culture that is thereupon filtered. This is also shewn by the following table giving the degrees of potency found.

Tests with	± or −+	+++	++++ or +++++	negative
F I	31	17	38	54
F II	17	19	38	64
F III	16	7	60	55

In the third filtrate one obtains a proportionally smaller number with + and +++, but a great number with ++++ and pl. clean.

In the following extract from the experiment register, only the fe ces and urine tests yielding positive results are included. All the negative, including those that yielded the granulated appearance only, are omitted.

		sion	Filtrate	Seedling	Seedling	Seedling	Filtrate	Seedling	Seedling	Seedling	Filtrate	Seedling	Seedling	Seedling
U	886	25-X-22	26-X-22	—	—	—	30-X-22	—	—	—	7-XI-22	—	+++	—
F	888	-	-	—	—	—	-	—	—	—	-	+?	+++	—
F	892	-	-	—	—	—	-	—	++	—	-	+++	—	—
U	892	-	-	-	—	—	-	—	+	—	-	—	—	—
F	894	-	-	—	—	—	-	—	—	—	-	—	gran.	+
F	900	-	-	—	—	—	31-X-22	—	—	—	-	?	+	—
F	918	27-X-22	28-X-22	++	+++	++++	-	—	+	—	-	++++	+++	—
U	918	-	-	++	+++	++++	-	—	+	—	-	++++	+++	+
U	921	28-X-22	29-X-22	—	—	—	1-XI-22	—	—	—	-	+	—	gran.
F	922	-	-	+	—	+	-	—	—	—	-	++++	—	gran.
U	922	-	-	+	+	—	-	—	+	—	-	—	—	—
F	923	-	-	—	—	+?	-	—	—	—	-	+++	++	gran.
F	925	-	-	+	+	+	-	—	+	—	-	+++	+	
U	927	-	-	+	++	+++	-	—	?	?	-	++++	—	—
F	928	-	-	+	+	+	-	—	—	—	-	—	—	—
U	929	-	-	—	+	—	-	—	—	—	-	—	—	—
F	930	-	-	+	+	+	-	-	—	—	-	—		—
U	934	-	-	++	++	++	-	+	+	+	-	++++	+	++
F	991	7-XI-22	8-XI-22	—	+++	gran.and spotted	17-XI-22	+++	++++	—	23-XI-22	—	++++	++++
F	1003	10-XI-22	12-XI-22	—	+	—	18-XI-22	—	—	—	-	—	—	—
F	1033	12-XI-22	13-XI-22	—	—	+++	17-XI-22	—	—	gran.	-	gran.	gran.	—
F	1035	-	-	+	+++++	+++	-	+++	+++	—	-	++++	++++	++++resist
F	1036	-	-	—	—	+	-	?	—	gran.	24-XI-22	++	—	—
F	1037	-	-	++	-	—	-	—	+++	—	-	—	—	—
U	1037	-	-	—	++	+	-	+++	—	—	-	+++	++++	++++resist
F	1039	-	-	++	+++	+	-	+++	+++	+resist.gran.	-	++++	++++	++++resist
U	1039	-	-	++	+++	+	-	+++	+++	+resist.gran.	-	++++	++++	++++resist
F	1040	-	-	—	-	+	-	++	+++	+ nibbled.	-	—	—	nibbled.
F	1068	17-XI-22	18-XI-22	+++	+++	—	24-XI-22	+++	+++	++++	29-XI-22	—	—	
U	1116	21-XI-22	22-XI-22	—	—	+++	29-XI-22	—	?	—	14-XII-22		—	—
F	1176	25-XI-22	26-XI-22	—	—	+	14-XII-22	—	—	—	20-II-23	—	—	—
U	1319	12-XII-22	14-XII-22	—	++	gran.	19-XII-22	—	—	—	-	—	—	—

	Suspension	I. Filtrate	1 Seeding	2 Seeding	3 Seeding	II. Filtrate	1 Seeding	2 Seeding	3 Seeding	III. Filtrate	1 Seeding	2 Seeding	3 Seeding
F 1046	14-XI-22	15-XI-22	—	—	—	25-XI-22	—	—	—	14-XII-22	gran.	gran.	++++
U 1046	-	-	—	—	—	-	—	—	—	-	gran.	gran.	++++
F 1047	-	-	?	—	—	-	—	—	—	-	gran.	gran.	++++
U 1047	-	-	?	—	—	-	—	—	—	-	gran.	gran.	++++
F 1049	15-XI-22	18-XI-22	—	—	+++	-	—	—	—	-	gran.	gran.	++++
F 1055	16-XI-22	-	—	—	—	-	—	—	—	-	gran.	—	+
F 1056	-	-	—	—	—	-	—	—	—	-	—	—	+
U 1056	-	-	—	—	—	-	—	—	—	-	—	—	+
F 1057	-	-	—	—	—	-	?	—	+	-	—	+	Pl. clean.
F 1060	17-XI-22	-	—	—	—	-	—	—	—	-	+	++	resist.
F 1061	-	-	—	—	—	-	—	—	—	-	—	—	+
U 1061	-	-	—	—	—	-	—	—	—	-	++	++	Pl. clean.
F 1075	18 XI-22	19-XI-22	—	—	—	24-XI-22	++	++	—	-	—	—	
F 1076	-	-	—	—	nibbled.	-	++	++	gran.	-	—	—	
U 1076	-	-	—	—	nibbled.	-	++	+++	gran.	-	—	—	
F 1077	-	-	—	—	—	-	(+)	+++	gran.	29-XI-22	—	—	
U 1077	-	-	—	—	+++	-	++	+++	gran.	-	—	—	
F 1078	-	-				-	++	++	gran.	-			
F 1079	-	-	—	—	—	-	+++	+++	gran.	-	—	—	
F 1080	-	-		—	+++	-	+++	+++	gran.	-	—	—	
U 1080	-	-	—	—	—	-	++	+++	gran.	-	—	—	
F 1081	-	-	—	—	—	-	+++	+++	gran.	-	—	—	
U 1082	-	-	—	—	—	-	+++	+++	gran.	-	—	—	
F 1088	20-XI 22	21-XI-22	—	—	—	29-XI-22	—	—	—	14-XII-22	—	—	+
F 1090	-	-	—	—	+++	-	—	—	—	16-XII-22	—	infected.	infected.
U 1091	-	-	—	—	—	-	—	—	—	-	—	—	++
F 1094	-	-	—	—	—	-	—	—	—	-	—	—	+
F 1095	-	-		—	—	-	—	—	—	-	—	—	++

	Suspension	I. Filtrate	1 Seeding	2 Seeding	3 Seeding	II. Filtrate	1 Seeding	2 Seeding	3 Seeding	III. Filtrate	1 Seeding	2 Seeding	3 Seeding
F 1097	21-XI-22	22-XI-22	—	—	+++	29-XI-22	—	—	—	16-XII-22	—	—	++
U 1097	»	»	—	—	—	»	—	—	—	»	—	—	++
F 1098	»	»	—	—	+ resist.	»	—	—	—	»	—	—	resist.
U 1098	»	»	—	—	—	»	—	—	—	»	—	—	resist.
F 1099	»	»	—	—	—	»	—	—	—	»	—	—	resist.
U 1099	»	»	—	—	+ resist.	»	—	—	—	»	—	—	—
F 1100	»	»	+++	++++	Pl. clean.	16-XII-22	—	—		»	+	—	Pl. clean.
U 1100	»	»	++++	++++	Pl. clean.	»	—	—		»	—	—	Pl. clean.
F 1102	»	»	—	—	—	»	—	—	+	»	?	—	++
F 1104	»	»	—	+	gran.	»	infected.	—	—	12-I-23	—	—	gran.
U 1104	»	»	—	+	gran.	»	—	—	gran.	»	—	—	—
U 1105	»	»	?	+	gran.	»	—	—	gran.	»	—	—	gran.
F 1108	»	»	—	—	+ resist.	»	—	—	gran.	»	—	—	gran.
F 1109	»	»	—	—	Pl. clean.	»	+++	++++	Pl. clean.	»	+	+++	Pl. clean.
F 1110	»	»	+	+	—	»	—	—	gran.	»	—	—	gran.
U 1132	23-XI-22	24-XI-22	—	—	—	9-I-23	—	—	—	»	—	+	gran.
F 1149	25-XI-22	26-XI-22	—	—	—	»	—	—	—	13-I-23	—	—	Pl. clean.
U 1149	»	»	++	++++	+ resist.	»	—	—	+++	»	—	—	—
U 1150	»	»	—	+	+ resist.	»	—	—	Pl. clean.	»	—	+	Pl. clean.
U 1152	»	»	++	+++	gran.	»	—	—	gran.	»	—	—	gran.
F 1154	»	»	+++	++++	+++++	»		—	Pl. clean.	»	—	—	Pl. clean.
F 1155	»	»	—	—	+++	»	—	—	—	»	—	—	—
U 1155	»	»	?	?	infected.	»	—	—	Pl. clean.	»	—	—	Pl. clean.
F 1156	»	»	+	—	—	»	—	—	—	»	infected.		—
U 1156	»	»	++++	++++	+ resist.	»	—	—	Pl. clean.	»	+	+	resist.
F 1157	»	»	++	+++	+++++	»	—	—	Pl. clean.	»	—	+	resist.
U 1157	»	»	+	+	+++++	»	—	—	Pl. clean.	»	—	—	resist.
F 1158	»	»	—	—	+++	»	—	—	—	»	infected.	infected.	?

	Suspension	I. Filtrate	1 Seeding	2 Seeding	3 Seeding	II. Filtrate	1 Seeding	2 Seeding	3 Seeding	III. Filtrate	1 Seeding	2 Seeding	3 Seeding
F 1160	25-XI-22	26-XI-22	—	—	?	9-I-23	—	—	+++	13-I-23	—	—	gran.
U 1160	»	»	—	—	?	»	—	—	+++	»	lacking.		—
U 1161	»	»	+	++	++++	»	—	—	Pl. clean.	»	—	+	resist.
U 1164	»	»	++	++	overgrown.	»	—	—	Pl. clean.	23-I-23	—	—	resist.
F 1165	»	»	+++	+++	»	»	—	—	Pl. clean.	»	—	—	resist.
U 1165	»	»	+++	++++	»	»	—	—	Pl. clean.	»	—	—	resist.
F 1167	»	»	—	—	»	»	—	—	gran.	»	—	+++	gran.
F 1226	28-XI-22	29-XI-22	—	—	—	27-XII-22	Pl. clean.	Pl. clean.	Pl. clean.	18-I-23	++	++	resist.
F 1229	»	»	—	—	—	»	+	++	++	»	—	—	—
U 1268	7-XII-22	11-XII-22	+++	+++	—	»	—	—	—	19-I-23	—	—	gran.
U 1299	10-XII-22	12-XII-22	+	++	Pl. clean.	»	—	—	Pl. clean.	20-I-23	—	+	resist.
U 1300	»	»	—	—	—	»	+++	++++	Pl. clean.	»	+	—	resist.
F 1302	»	»	—	—	Pl. clean.	»	++++	Pl. clean.	Pl. clean.	»	+++	++++	resist
U 1302	»	»	++	++	Pl. clean.	»	+	++	Pl. clean.	»	+	—	resist.
F 1303	»	»	—	—	+	»	—	—	—	»	—	—	—
U 1305	»	»	—	—	Pl. clean.	»	++	++	Pl. clean.	«		—	resist.
F 1306	»	»	+++	+++++	Pl. clean.	»	+++	+++	Pl. clean	»	—	—	resist.
U 1306	»	»	+	—	Pl. clean.	»	+	+	Pl. clean.	»	lacking.		
U 1323	12-XII-22	14-XII-22	—	—	+	»	—	—	—	23-I-23		—	gran.
U 1325	»	»	—	—	—	»	—	+	gran.	»	—	—	gran.
F 1327	»	»	—	—	Pl. clean.	»	++	++	Pl. clean.	»	++	+++	Pl. clean.
U 1327	»	»	—	+	Pl. clean.	»	++	++	Pl. clean.	»	+	+	Pl. clean.
F 1331	»	»	—	—	+	»	—	—	gran.	»	—	—	gran.
F 1336	13-XII-22	15-XII-22	—	—	—	28-XII-22	+	—	gran.	24-I-23	—	—	gran.
F 1352	15-XII-22	17-XII-22	++	++++	overgrown.	»	+++	++++	Pl. clean.	26-I-23	++	+++	Pl. clean.
U 1352	»	»	++++	Pl. clean.	»	»	+++	++++	Pl. clean.	»	—	—	—
U 1354	»	»	—	—	++	»	—	—	gran.	»	—	—	gran.

	Suspension.	I. Filtrate	1. Seeding.	2 Seeding.	3 Seeding.	II. Filtrate	1 Seeding.	2 Seeding.	3 Seeding.	III. Filtrate	1 Seeding.	2 Seeding.	3 Seeding.
U 1373	18-XII-22	20-XII-22	—	—	++	27-XII-22	—	—	—	8-I-23	—	—	—
F 1374	»	»	+	++	gran.	»	—	—	—	»	—	—	—
F 1376	19-XII-22	»	—	—	Pl. clean.	»	+	+	Pl. clean.	»	+	++	Pl. clean.
U 1376	»	»	—	—	—	»	++	overgrown.	++++	»	+	++	+++
F 1397	21-XII-22	22-XII-22	—	—	—	»	—	—	—	»	—	—	+++
U 1397	»	»	—	—	+++	»	—	—	—	»	—	—	—
F 1401	»	»	—	—	—	»	—	—	+	»	—	—	—
U 1401	»	»	—	—	Pl. clean.	»	—	—	Pl. clean.	»	—	—	Pl. clean.
F 1402	»	»	—	—	+	»	—	—	—	»	—	—	—
U 1433	27-XII-22	28-XII-22	+	—	Pl. clean.	11-I-23	gran.	—	—	26-I-23	—	—	—
U 1434	»	»	+	—	Pl. clean.	»	+++	+++	resist.	»	+++	+++	Pl. clean.
U 1435	»	»	—	—	+++	»	—	+	gran.	»	—	—	gran.
F 1443	28-XII-22	2-I-23	—	—	++	»	—	—	—	»	—	—	gran.
F 1447	»	»	—	—	—	»	++	?	gran.	»	—	—	—
U 5	2-I-23	4-I-23	+	+	overgrown.	12-I-23	—	—	resist.	28-I-23	—	+++	Pl. clean.
F 6	»	»	—	—	++	»	—	—	—	»	—	—	—
U 25	7-I-23	8-I-23	—	—	—	24-I-23	—	+++	gran.	»	—	—	—
U 28	»	»	—	—	++	»	—	—	gran.	»	—	—	resist.
F 75	13-I-23	16-I-23	gran.	resist.	—	26-I-23	—	—	—	»	—	—	—
F 86	16-I-23	19-1-23	++	—	—	»	+++	++++	++++	»	resist.	resist.	—
U 86	»	»	—	resist.	resist.	»	+++	++++	++++	»	resist.	resist.	—
U 102	22-I-23	24-I-23	—	—	—	»	+++	resist.	resist.	»	resist.	resist.	resist.
F 103	»	»	—	+	resist.	»	gran.	resist.	—	»	resist.	resist.	resist.
U 103	»	»	+++	+++	resist.	»	gran.	resist.	resist.	»	resist.	gran.	gran.
F 104	»	»	—	++	—	»	—	—	—	»	—	—	—
U 104	»	»	+	+	resist.	»	+++	++++	resist.	»	resist.	resist.	resist.
U 107	»	»	—	—	gran.	»	+++	resist.	gran.	»	resist.	resist.	resist.
U 109	»	»	—	++	gran.	»	++	+++	gran.	»	resist.	gran.	gran.

Farthest to the left are placed the serial numbers. F stands for feces and U for urine specimens; then follows the date at which the specimens arrived and were suspended; and finally when the filtrations and seedings took place.

The subject of these investigations was, as already mentioned a paratyphoid epidemic at Lemvig in Jutland and a typhoid epidemic at Bogense on Funen.

The epidemic at Lemvig comprised 32 cases all yielding positive Widals reaction against paratyphoid. All these could be traced to the same source of infection viz. infected milk from a dairy.

The investigations concerning this epidemic were not initiated until after the majority of the patients were without fever, for which reason they were not suitable for the elucidation of the matter in question.

The epidemic at Bogense, comprised 28 cases of typhus with positive Widal reaction. In respect to these, the investigations were initiated as soon as the patients were admitted to hospital and even if the forwarding of the specimens left much to be desired as regards regularity, it, however, proved that from the material collected, quite extensive conclusions could be drawn with regard to the problems to be solved.

Lemvig Epidemic.

Feces and urine specimens from 32 patients were examined.

Bacilli were found in 12 patients (37%)
Lysin was - - 19 — (59%)

In all 144 feces and 144 urine specimens were examined for bacilli and 116 feces and 116 urine specimens were examined for lysin.

Bacilli were found in 14 specimens of feces (10%)
— - - - 13 — - urine (2%)

Lysin was found in 19 specimens of feces (16%)
- - - - 10 — - urine (9%)

The granulated appearance without plaques was found in 12 feces and 2 urine tests.

The Bogense Epidemic.

Feces and urine specimens from 28 patients were examined.

Bacilli were found in 7 patients (25%)
Lysin was - - 26 — (93%)

In all 218 feces and 218 urine specimens were examined.

Bacilli were found in 6 feces specimens (2.7%)
— - - - 5 urine — (2.3%)

Lysin was found in 44 feces specimens (21%)
- - - - 40 urine — (18%)

The granulated appearance alone was found in the examination of 36 feces specimens (17%) an 42 urine specimens (19%).

Below the figures are given collectively shewing the percentage:

		Lysin	Bacilli
Lemvig.	patients	59%	37%
	feces specimens	16%	10%
	urine —	9%	2%
Bogense.	patients	93%	25%
	feces specimens	21%	2.7%
	urine spcimens	18%	2.3%

During the Lemvig epidemic lysin was found in 59% of the patients. That these figures are so much lower than those of the Bogense epidemic is certainly not due to lysin being more rarely found in paratyphoid patients, but to the fact, that the investigations were initiated so late during this epidemic. Hereby fewer specimens were produced from the individual patients and then only during a single phase of the disease, (the apyretic).

Lysin is demonstrated far more frequently than bacilli. Greatest is the difference in the case of the Bogense epidemic where lysin is found in respect to the feces specimens 7, and the urine specimens 6 times more frequently than bacilli. That the difference is greatest in the Bogense epidemic may probably be attributed to the fact that here a thyphoid epidemic is concerned. Probably the typhoid bacilli

decompose far more rapidly than those of paratyphoid and as the specimens as a rule, were 24 hours or more in transit it must be presumed that the bacilli had decomposed. To this be added, that the technique for the demonstration of bacilli in feces yields figures, that must be regarded as being far below the number of cases in which they actually are present. But even if we reckon that the bacilli were not demonstrated in all cases where they actually were present, it would scarcely be right to presume that they had been present in 6 or 7 times the number of cases in which they were discovered. A lysin that dissolves typhoid bacilli may consequently, undoubtedly be found in the feces where there are not or have not recently been typhoid bacilli. The explanation to this is probably that the lysins may be, what might be termed »polyvalent«. By this is meant, that a lysin that dissolves typhoid bacilli, and at the same time dissolves coli bacilli and is capable of multiplying at the expense of these, while simultaneously retaining its capacity to dissolve the typhoid bacilli. It has previously been said that as we can also find a lysin that, as far as we know, has never been in contact with typhoid bacilli, but that dissolves them nevertheless, it is evident that the demonstration of a lysin in feces will be of no use in the diagnosis of any certain disease, or for the demonstration that difinite bacilli are or have been present in the material examined.

It is of great interest to observe in how many cases lysin is demonstrated simultaneously in urine and feces.

Lysin is demonstrated in 50 specimens of urine. In the case of 30 of these, it was simultaneously demonstrated in specimens of feces taken at the same time as the urine. This figure is high and points to the fact that when lysin is found in the urine, it will likewise be found in feces.

If, in the urine, typhoid or paratyphoid bacilli are found, it must be taken into account, that these are more easily demonstrable therein than in feces. This will naturally, in a heigh degree, be the case, when one is able to spread from recently passed urine or urine taken with a catheter. In such urine several bacterial species will rarely be found, and should typhoid or paratyphoid bacilli be present, these will be more easily demonstrable than in feces.

From this argument one can take for granted, that in urine in

which typhoid or paratyphoid bacilli are not to be found, they, in a relatively large number of cases, are not present.

In my material of 334 specimens of urine, bacilli have been demonstrated in 8. If we make a calculation of probabilities and presume that they may have been present in 3 times the number of cases, the figure is 24. In the same 334 specimens of urine, lysin has been demonstrated in 50 cases. (In one case only, were lysin and bacilli demonstrated simultaneously). If we subtract from these 50, the 24 cases in which there are presumed to have been bacilli present, there are 26 specimens of urine left in which lysin is found, but no bacilli. It would appear probable that the lysin in these cases is conveyed to the urine by the blood and probably has been secreted by the kidneys.

If this be the case, we are approaching the question as to where in the organism the lysin is formed.

According to d'Herelle's conception the answer to this is, that the lysin is conveyed to the body from outside and multiplies where bacilli are present. This may be correct, and in such a case the lysin must be presumed to be conveyable by the blood from the spot where it is formed to the kidneys and other organs, through which it is secreted. But there still remains the possibility that it is formed at some other spot or spots in the organism than where the bacilli are to be found, e. g. in the blood or in some gland or other, and from there is conveyed to the intestine and to the kidneys. Bacilli are certainly found in the blood too, at a certain stage of the disease, but they cannot be presumed to be present during the whole period in which lysin is demonstrable in the patient.

The problem as to where in the organism lysin is formed is quite immenent, but even if it is not solved by these researches, it would appear, as I have pointed out, that through the above circumstances, there is a possibility of demonstrating that lysin is something more universally distributed, that not alone can be demonstrated where bacilli are or have recently been present; in other words, that it cannot be a parasite that is only found where bacilli are present, and at their expense. At any rate, it appears to me, that such an extensive demonstration of lysin, without bacilli, tells against the

theory, that it is an infectious matter to the bacilli in the sense meant by d'Herelle.

As I have already said, lysin cannot be used for diagnostic purposes. But, on the other hand, one must ask whether the lysin could interfere with the demonstration of the bacilli. This might be imagined to occur, either by their dissolving the bacilli, or by their changing the appearance of the bacillary colonies, in such a manner, as to resemble colonies of non-pathogenic bacteria.

This must be regarded as very probable. The colonies of the resistant bacteria have an appearance that in a high degre deviates from what is demanded of a colony to be accepted as a typhoid or paratyphoid colony. They are frequently found as large flat colonies with dull opalescent lustre, as opposed to the clear bluish stilliform typhoid and paratyphoid colonies. Researches on this have not been made, but would doubtlessly yield interesting results, and tend to increase the incomprehensively low number of cases in which the bacilli, are found, even by repeated examinations of both feces and urine during the whole course of the disease.

If we then turn to the relation of the lysin to the actual course of the disease, this is best illustrated by the temperature curves. As a matter of fact namely the condition of a typhoid patient, when cases of collapse, with low or subnormal temperature, are excluded, in everything essential depends upon the height of the temperature.

With regard to the Lemvig epidemic, the researches were initiated so late, i. e. after almost all the patients were without fever, that no conclusions could be drawn, excepting that the lysin was demonstrated at the apyretic stage with the frequency stated.

In respect to the Bogense epidemic the researches were initiated as soon as the patients were admitted to hospital; but unfortunately were not carried out with the projected frequency, owing to the specimens being forwarded somewhat irregularly.

I have divided the patients into 4 groups:

Group I. Consisting of 12 patients in whom lysin was demonstrated in the febrile stage and at least 9 days before the temperature had become normal.

Group II. Consisting of 10 patients in whom the lysin was, at the earliest, demonstrated simultaneously with the fall in temperature.

Group III. That coincides with the Lemvig epidemic, the Lysin being demonstrable for the first time at the apyretic stage. This group consists of 7 patients.

Group IV. The negative, of which there were 3 cases.

GROUP I.

In the following summary of 12 patients, the patients initials are placed to the extreme left, then the day of illness when lysin was first demonstrated in the patient and finally to the extreme right the number of days elapsing before the temperature became normal. The number of + states the degree of potency of the specimen in question.

Patients initials	Day of illness on which lysin first demonstrated	Temperature normal after
1. H. P. R.	10 +++++	18 days
2. L. H. K. M. R.	10 +++++	19 »
3. M. R.	8 ++	14 »
4. L. J.	18 ++	13 »
5. H. A.	6 +	19 »
6. A. D. M. J.	15 +++	12 »
7. Aa. J.	7 +++	24 »
8. K. Mr. J.	7 ++	9 »
9. K. Mg. J.	12 +++++	36 »
10. C. K.	11 +++	20 »
11. M. K. K. M.	17 ++	22 »
12. H. C. R.	9 +++++	22 »

From these figures, that merely state the earliest point of time at which the lysin was demonstrated, and by the temperature curves (see pag. 157) on which in many cases one will find potent lysins demonstrated repeatedly during the highly febrile stage, it will be seen that the lysin is not, as d'Herelle contends, a phenomenon that occurs in immediate connection with, and as the cause of the fall in temperature.

Patient No. 1 H. P. R. may be regarded as especially characteristic. Here a potent lysin was demonstrated from admittance to the 11th day

of illness, and thereupon during the whole illness. Nevertheless th temperature did not fall until the 24th—26th day of illness or 15 day after the lysin for the first time was demonstrated. In patient No. K. M. J. a potent lysin was demonstrated on the 12th day of illnes and the temperature did not fall until 36 days later.

GROUP II.

This group comprises 10 patients in which the lysin was demon strated simultaneously with the fall in temperature, but not before.

As regards 7 of these patients the circumstances are that they were not examined for lysin before the temperature began to fall. These patients, therefore, may well have had lysin from the commencemen of the illness, as was the case with those in group I. In respect t the 3 remaining patients, they were examined twice with negativ result during the febrile stage of the illness, whereupon they were examined 3 times with positive result simultaneously with the fal in temperature. In 4 of the negative tests, however, the granulate appearance of the culture was observed, thus these 3 patients may perhaps, have had the lysin during the whole illness.

GROUP III.

The patients in this group are as those in group II; inasmuch as 3 of them were not examined before the fall in temperature, whereas the rest are doubtful, a number of the specimens having possibly contained lysin.

The results from group II and III do not, therefore, invalidate th result from group I.

The fact that lysin is found in patients from the very beginnin of the disease thus shews, that there can be no causative connectio with the fall in temperature, not, in any case, in the manner con tended by d'Herelle.

In other respects also this result is of significance. If d'Herelle' theory were correct, it would be a considerable support for the thera peutic applicability of the lysin, from the reasoning, that if lysi were the cause of the fall in temperature, it ought to be possible t produce such a fall by administering to the patient a potent lysi immediately the disease was ascertained.

Here, however, we have a material, consisting of 12 patients in whom lysin was present, in some cases even in a very potent form, at an early stage of the disease, and yet the course of the disease did not deviate in any respect from that ordinarily met with. The temperature persisted for from 19 to 36 days after the lysin was demonstrated; in one case, however, for only 9 days.

If we return to the point of departure of these researches, the questions posed can be answered as follows:

The lysin demonstrated by d'Herelle is found in almost all the typhoid patients examined viz. in 93 %. In the paratyphoid patients, who were only examined at the apyretic stage, the lysin was demonstrated in 59 %.

The lysin may be demonstrated both in the stools and in the urine, both long before and also after the fall in temperature and may thus be presumed not to be in any causative relationship to it.

Consequently there is but little probability, that the course of a disease can, at an early stage, be influenced by administering to the patient a lysin of this description.

The effect obtained by treatment with lysin may be presumed to be due to a vaccination effect that possibly is augmented by the particular manner in which the bacilli are dissolved.

THE LYSIN TREATMENT OF BACILLI CARRIERS.

In the study of epidemic infectious diseases the question of bacilli carriers will invariably rank as an important one. If we could find a way to rid bacilli carriers of their bacilli, we should have made great progress in the combating of epidemics. Unfortunately we are still powerless to treat bacilli carriers and in this place I do not enlarge upon all the experiments carried out to procure such a means.

d'Herelle's bacteriophages presented a medium that, a priori, raised great expectations, all the more so when Kabeshima's rabbit experiment promised that the result would be favourable.

Before attempting to treat bacilli carriers with lysin, it was my intention to test Kabeshima's experiments. I was also successful in producing some rabbits typhoid carriers by injecting typhoid bacilli

into the gall bladder. Before they were treated with lysin, however, they had to be killed as they presented too great a danger as a source of infection.

In connection with these experiments a number of experiments were carried out to ascertain what effect intravenous injections of lysin had on healthy rabbits. These experiments shewed that the intravenous injection of lysin produced the formation of the same amount of agglutinin as the injection of the same amount of bacterial culture.

Before communicating the course and result of the treatment of a bacillary carrier, some few remarks regarding the difficulties in the estimation of the effect of the lysins would be to the point.

The first bacillary carrier to be treated was a woman, K. M., who was employed as a kitchen hand. For 10 months she had secreted paratyphoid bacilli and these were, as a rule, found in pure culture in her stools. At request I endeavoured to produce a lysin that dissolved these bacteria. The difficulties in doing so I have already mentioned and in this case almost two months elapsed before I was successful in discovering a lysin that was suitable for the treatment. When finally I was successful in doing so, I examined, to ascertain whether she was still a bacillary carrier, a series of stools. Whereas she previously regularly had had pure culture of paratyphoid bacilli in her feces, none was now found in the stools examined. The same happened with another bacillary carrier M. H. who, however, had only secreted bacilli for a short time. If one had commenced the treatment of these two »at the right moment« the conclusion that the lysin had brought about their cure would have been all but obvious. Now, however, we were prevented from coming to such conclusions and the next case, in which the treatment was accomplished, shewed with all the desired distinctness that the presence of a lysin with a potenty dissolving capacity against the bacteria secreted, did not prevent their continued secretion.

The patient was a 26 year old cowman, whose work consisted of attending to and milking cows. He was admitted to Ringsted hospital on 13. VIII. 23, diagnosed as paratyphoid fever. On admission he had been ill for about a week with violent headache, severe dedolations, rise in temperature to between 38^0—39^0, but no diarrhoea

or vomiting. On admission his temperature was 37.9° and in the course of a couple of days became normal. Widals reaction on Aug. 13th 23 was positive for paratyphoid 1/50. In the feces on Aug. 15th paratyphoid bacilli were found. The only treatment he received was treatment with lysin and this was not begun until he was recognised as a bacilli carrier. This happened when for 2 months he had had pure culture of paratyphoid in his stools. At that juncture I was asked to attempt a treatment with lysin. Paratyphoid bacilli isolated from his stools was tested against the following lysins in my lysin collection:

Seeding.

	Immediately	After 4 hours	After 20 hours
Coli d'H.	—	—	—
» CB.....	—	—	—
» Lanz	—	—	—
» 873.....	—	—	—
» Tietz	—	—	—
Typhoid T3	++	+++	+++++
» 126	—	—	—
» 116	—	++	+++
Paratyphoid 1022..	+++	++++	++++++
» 1082..	+++	++++	++++
» K. M..	++	+++	++++
Dysenteriae S. pn..	—	—	—
» S. I. ..	—	—	—
Staph. d'H.	—	—	—
» cer. alb.....	—	—	—

From these paratyphoid strain number 1022 was chosen as being the strongest. From this a large quantity was prepared by filtration of a 24 hour old lysin culture. After filtration, heating to 56° for 3/4 of an hour. On measuring the prepared lysin it was found to contain 2000 million lysin units per c. c.

Before the lysin was despatched I made two injections on myself, one of 1/2 c. c. and the other of 2 c. c. Except for a slight headache they produced no reaction whatever. It must be remarked that some time before I had been inoculated against paratyphoid.

The course of treatment and the result are as follows:

13. VIII. Admitted to Ringsted hospital. Widal positive for paratyphoid $^{1}/_{50}$.

15. VIII. Temperature normal. Feces + B. paratyph.

$^{29}/_{8}$, $^{7}/_{9}$, $^{18}/_{9}$, $^{25}/_{9}$, $^{3}/_{10}$, $^{11}/_{10}$, $^{23}/_{10}$, $^{30}/_{10}$, $^{6}/_{11}$, $^{7}/_{11}$, $^{8}/_{11}$, $^{11}/_{11}$, $^{13}/_{11}$, $^{16}/_{11}$ and $^{18}/_{11}$ B. paratyph. in feces.

$^{19}/_{11}$: 0.15 c. c. lysin administered subcutaneously. Feels well. Tp. 37.5/36.8.

$^{20}/_{11}$: Tp. 37.5/36.8. Apart from slight headache no inconvenience after injection. Today no headache.

Lysin administered subcutaneously 0.3 c. c.

Feces: + B. paratyph.

$^{21}/_{11}$: Tp. 37.6/37. Yesterday some headache, also today, no injection is therefore given.

^{22} $_{11}$: Tp. 37.4/36.8. Feels well.

Lysin administered subcutaneously 0.50 c. c.

Feces: + B. paratyph.

$^{23}/_{11}$: Tp. 37.4/36.8. Again headache.

$^{24}/_{11}$: Tp. 37.3/36.8. Headache slightly abated, complains of diffuse pains in abdomen.

$^{25}/_{11}$: Tp. 37.4/36.8. Headache more violent, no pains in abdomen.

Feces: + B. paratyph.

$^{26}/_{11}$: Tp. 37.4/36.8. Feels well.

Lysin administered subcutaneously 0.60 c. c.

$^{27}/_{11}$: Tp. 37.4/36.9. Slight headache, otherwise feels well.

Feces: + B. paratyph.

$^{28}/_{11}$: Tp. 37.4/36.9. Feels well.

Lysin administered subcutaneously 1 c. c.

$^{29}/_{11}$: Tp. 37.4/37. Again headache.

Feces: + B. paratyph.

$^{30}/_{11}$: Tp. 37.6/36.9. Feels well.

Lysin administered subcutaneously 1.5 c. c.

$^{1}/_{12}$: Tp. 38/36.9. Very severe pains and distinct tenderness in region of gall bladder.

$^{2}/_{12}$: Tp. 37.6/36.7. Fairly severe pains and tenderness unchanged.

Feces: + B. paratyph.

The pains and tenderness disappeared in the course of a few days, temp. also became normal.

$^{2}/_{12}$, $^{4}/_{12}$ and $^{6}/_{12}$. Feces: + B. paratyph.

$^{7}/_{12}$: Lysin administered subcutaneously 1.5 c. c.
Without reaction.

$^{9}/_{12}$: Feces: + B. paratyph.

$^{11}/_{12}$: Feces: + B. paratyph.

$^{12}/_{12}$: Lysin administered subcutaneously 1.5 c. c.
Without reaction.

$^{13, 16, 21, 23, 26, 28, 30}/_{12}$, $^{1, 5, 15, 21, 26}/_{1}$ 1924 and $^{8, 15, 21}/_{2}$ found in feces:
B. paratyph.

$^{23}/_{2}$: Lysin administered per os 1.5 c. c.

$^{24}/_{2}$: Lysin administered per os 3.0 c. c.

$^{25}/_{2}$: Feces: + B. paratyph. + lysin of med. potency.
Lysin administered per os 6.00 c. c.

$^{26}/_{2}$: Feces: + B. paratyph. + potent lysin.
Lysin administered per os 12.0 c. c.

$^{27}/_{2}$: Lysin administered per os 24.0 c. c.

$^{28}/_{2}$: Feces: + B. paratyph. + potent lysin.
Lysin administered per os 24.0 c. c.

$^{29}/_{2}$: Lysin administered per os 26.0 c. c.

$^{2}/_{3}$: Feces: + B. paratyph. + potent lysin.

$^{6}/_{3}$: » + » » + » »

$^{15}/_{3}$: » + » » + » »

$^{20}/_{3}$: » + » » + » »

$^{22}/_{3}$: » + » » + » »

$^{24}/_{3}$: » + » » + » »

$^{26}/_{3}$: » + » » + » »

$^{27}/_{3}$: » + » » + » »

$^{28}/_{3}$, $^{7}/_{4}$, $^{22}/_{4}$, $^{28}/_{4}$, $^{14}/_{5}$, $^{20}/_{5}$, $^{28}/_{5}$, $^{3}/_{7}$, $^{18}/_{9}$ 1924 in feces B. paratyph.

At one juncture ($^{24}/_{3}$ 1924) only a few paratyphoid bacilli were found. At all other examinations there was almost a pure culture of these.

In the stools examined resistant paratyphoid bacilli were never found.

The paratyphoid bacilli used for the lysin test were invariably such as were cultivated from the latest specimen of feces from the patient.

From these researches it will be seen that at all examinations of feces (61 in all) undertaken during 13 months after the temperature had become normal paratyphoid bacilli were demonstrated; that large doses of lysin administered both subcutaneously and per os had no effect on the excretion of the bacteria, and that the presence in the feces of a potent lysin against the secreted bacteria, even throughout a protracted period did not succeed in causing the secretion to cease, neither did it have any influence on the quantity of the secreted bacteria.

The reactions that the lysin caused were headache and diffuse pains in the abdomen. Whether the pains and tenderness in the region of the gall bladder present at one period were caused by the injections of lysin could not be decided. The fact that 2 subsequent injections of the same volume did not produce effects of a similar nature, tell against this being the case. On the other hand it cannot be denied that the fact, that during the treatment such serious symptoms appeared, warns us to be cautious and not undertake this treatment with out-patients.

Should the result of the treatment undertaken be discussed there might be the necessity of making the following remark.

One single case is not sufficient to warrant the rejection of a treatment as promising as this. Naturally the treatment of several cases would be of great significance; but the fact that in one single bacilli carrier, simultaneously in the feces, pure cultures of the pathogenic bacteria and a lysin with a potenly lytic capacity against these, may be found, has the effect of making one somewhat sceptical with regard to such treatment.

It is perhaps a mistake to begin with such small doses. This is, however, difficult to avoid it being impossible beforehand to know whether it is justifiable to begin with large ones.

SUMMARY

1. A critical review of the literature arranged according to the main problems of the topic is given.
2. At the examination of 13 patients lysin is found in 6 of them.
3. On examining the lysins found, it is ascertained that they are unspecific with regard to the bacterial species examined, in the sense, that a given lysin dissolves bacteria of widely differing species, but that their action within one and the same species is, to a certain degree, specific; a given lysin dissolving some strains, but not others.
4. A technique is submitted for the demonstration of lysin. A main factor in it being the repeated spreading from the mixture of the material examined and the bacteria within the first 3 hours.
5. At the examination of the lytic action on various substrata it proved that the medium was relatively a matter of indifference, if only they afford favourable growth conditions for the bacteria upon which the lysin is to develop.
6. The lytic action is independant of the presence of the nitrogen.
7. The optimum temperature for the lytic action was between 37^0 and 40^0. At 45^0 there is no lytic action. This persists but, in a lesser degree at temperatures as low as 20^0.
8. The optimum reaction of the substrata was, in respect to the lysins examined, between the p_H figures of 7 and 8. p_H figures under 6 and over 8 yielded but slight increase of lysin.
9. Three factors are given as a gauge for the potency of a lysin, the reaction figure, the reaction velocity and the reaction maximum, and a technique is submitted for the measuring of these.
10. By means of detailed experiments on the course of the process of dissolution it is demonstrated:
    a) that an alternating action takes place between the lysin and the bacteria that can be observed for up to 52 hours,

b) that this alternating action, that can be demonstrated by frequent spreadings during the course of the process, does not depend upon the varying quantities of lysin, but upon the fluctuating susceptibility in the bacteria present at every juncture,

c) that the quantity of lysin increases steadily to maximum where it remains,

d) that the quantity of bacteria capable of multiplication rises during the first phase, in the same manner as though no lysin were present and reaches its maximum before the concentration of lysin has reached its maximum,

e) that the increase in the concentration of lysin may thus be due to the decay of bacteria.

11. By the seeding experiments it was demonstrated that the proportion between the number of resistant and susceptible bacteria continually changes during the course of the process of dissolution.

12. By a microscopic examination it is elucidated

a) how the dissolution of the individial bacteria occurs both in diluted and in undiluted lysin,

b) that the resistant bacteria are already present in the culture and do not occur as later generations of susceptible bacteria, and

c) how plaques are formed.

I was, however, unsuccessful in discovering by microscopic examination the reason for the localized action of the lysin on solid substrata.

13. I was not successful, at will, in producing lysins by means of Borchardt's technique, whereas a modification of this yielded good results.

14. In typhoid patients lysin was demonstrated against the pathogenic bacteria in 93 % of the cases (28 patients). In paratyphoid patients, in the apyretic stage, lysin was demonstrated against the pathogenic bacteria in 59 % of the cases (32 patients).

15. Lysin against the pathogenic bacteria can be demonstrated in the stools and urine, both long before, and long after the fall in temperature, and cannot thus be presumed to stand in any causitive relationship to this.

16. There is, consequently, no probability that the course of a disease may be influenced by administering lysin to the patient at an early stage of the disease.
17. The effect obtained by treatment with lysin, may be presumed to be a vaccination effect.
18. An examination of a paratyphoid bacillary carrier treated with lysin was undertaken.

At all examinations of feces (total 61) undertaken throughout 13 months, after the temperature had become normal, paratyphoid bacilli were demonstrated. Even large doses of lysin administered both per os and subcutaneously had no effect upon the excretion of pathogenic bacteria.

The presence of a potent lysin in the intestine itself for a protracted period does not arrive at causing the excretion of bacteria to cease, neither has it any influence on the quantity of the bacteria excreted.

During treatment symptoms of a local reaction in the gall bladder occurred, which tends to suggest that such treatment is not free from danger.

RÉSUMÉ

1. Der gives en oversigt over literaturen ordnet efter emnets hovedproblemer.
2. Ved undersøgelse af 13 patienter findes lysin hos 6 af disse.
3. Ved undersøgelse af de fundne lysiner findes, at disse er uspecifikke med hensyn til de undersøgte bakteriearter i den forstand, at et givet lysin opløser bakterier af vidt forskellig art, men at deres virkning indenfor een og samme art til en vis grad er specifik, idet et givet lysin opløser nogle stammer men ikke andre.
4. Der angives en teknik til paavisning af lysin. En hovedbetingelse for denne er det, at der spredes gentagne gange fra blandingen af det undersøgte medium og bakterierne indenfor de første 3 timer.
5. Ved undersøgelsen af lysinvirkningen paa forskellige substrater viste det sig, at disses art er relativ ligegyldig, naar blot de yder gode vækstbetingelser for de bakterier, paa hvilke lysinet skal udvikle sig.
6. Lysinvirkningen er uafhængig af tilstedeværelsen af ilt.
7. Temperaturoptimum for lysinvirkningen ligger mellem 37^0 og 40^0. Ved 45^0 er der ingen lysinvirkning. Denne holder sig omend i ringe grad ved temperaturer ned til 20^0.
8. Substraternes optimale reaktion laa for de undersøgte lysiners vedkommende mellem p_H-værdierne 7 og 8. p_H-værdier under 6 og over 8 gav kun ringe lysinforøgelse.
9. Som maal for et lysins styrke angives 3 faktorer, reaktionstallet, reaktionshastigheden og reaktionsmaximum, og der angives en teknik til maaling af disse.
10. Gennem udførlige forsøg over opløsningsprocessens forløb paavises,
    a) at der foregaar en vekselvirkning mellem lysin og bakterier, der lader sig følge i indtil 52 timer,
    b) at denne vekselvirkning, der lader sig paavise ved hyppige

spredninger under processens forløb, ikke beror paa vekslende mængder lysin, men paa vekslende modtagelighed hos de paa ethvert tidspunkt tilstedeværende bakterier,

c) at lysinmængden stiger jævnt til maximum og derefter forbliver maximal,

d) at mængden af formeringsdygtige bakterier under processens første fase stiger paa samme maade, som hvis der ikke var lysin tilstede og naar sit maximum inden lysinmængden er blevet maximal,

e) at forøgelsen af lysinmængden saaledes kan skyldes henfald af bakterier.

11. Ved spredningsforsøg paavises, at forholdet mellem antallet af resistente og modtagelige bakterier stadig forandres under opløsningsprocessens forløb.

12. Gennem en mikroskopisk undersøgelse klarlægges,

a) hvorledes opløsningen af de enkelte bakterier foregaar, dels i fortyndet, dels i concentreret lysin,

b) at de resistente bakterier paa forhaand er tilstede i kulturen og ikke fremkommer som senere generationer af modtagelige bakterier,

c) hvorledes pletterne dannes.

Derimod lykkes det ikke ved mikroskopisk undersøgelse at erkende aarsagen til lysinets lokaliserede virkning paa faste substrater.

13. Det lykkedes ikke vilkaarligt at fremstille lysiner ved hjælp af Borchardts teknik, hvorimod en modifikation af denne gav gode resultater.

14. Hos tyfuspatienter paavises lysin mod de pathogene bakterier i 93 % af tilfældene (28 patienter). Hos paratyfuspatienter i det afebrile stadium paavises lysin mod de pathogene bakterier i 59 % af tilfældene (32 patienter).

15. Lysin mod de pathogene bakterier kan paavises i afføringer og urin saavel længe før som længe efter temperaturfaldet og kan saaledes ikke formodes at staa i noget aarsagsforhold til dette.

16. Der er derfor ikke nogen sandsynlighed for, at man kan paavirke sygdommens forløb ved paa et tidligt tidspunkt af sygdommen at bibringe patienterne lysin.

17. Den virkning, som er opnaaet ved behandling med lysin, maa formodes at være en vaccinationsvirkning.

18. En paratyfusbacilbærer er behandlet med lysin.

Ved alle undersøgelser af fæces (ialt 61) foretaget gennem 13 maaneder, efter at temperaturen var blevet normal, er paavist paratyfusbaciller. Selv store doser lysin givet saavel per os som subcutant havde ikke nogen indflydelse paa udskillelsen af de pathogene bakterier.

Tilstedeværelsen af et kraftigt lysin i tarmen selv gennem længere tid formaar ikke at bringe udskillelsen af bakterier til ophør, ej heller har den nogen indflydelse paa mængden af de udskilte bakterier.

Under behandlingen optraadte symptomer paa en lokal reaktion i galdeblæren, hvilket lader formode, at en saadan behandling ikke er farefri.

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C. r. soc. biol. = Comptes rendus de la société de biologie.
W. kl. W. = Wiener klinische Wochenschrift.
Med. Kl. = Medicinische Klinik.
Zeitschr. f. Imm. = Zeitschrift für Immunitätsforschung.
J. of Bact. = Journal of Bacteriology.
Kl. W. = Klinische Wochenschr.
Proc. of Soc. f. exp. Biol. a Medc. = Proceedings of Society for experimental Biology and Medicine.
Schw. med. W. = Schweizerische medizinische Wochenschrift.
C. f. Bakt. = Centralblatt für Bakteriologie, Parasitologie u. s. w.
J. of exp. Medc. = Journal of experimental Medicine.
D. M. W. = Deutsche medizinische Wochensbrift.
M. m. W. = Münchener medizinische Wochenschrift.
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TEMPERATURE CURVES

DESCRIPTION OF THE TEMPERATURE CURVES

Uppermost the no. and name of the patient.

The row of figures under the name shews the dates.

In the first transversal column the result of bacillary examination is shewn, + denoting that bacilli have been found, — that none have been found.

In the next transversal column, the result of examination for lysin is given, — shews that no lysin have been found, + denotes lysin found. Number of + shews the potency of the lysin.

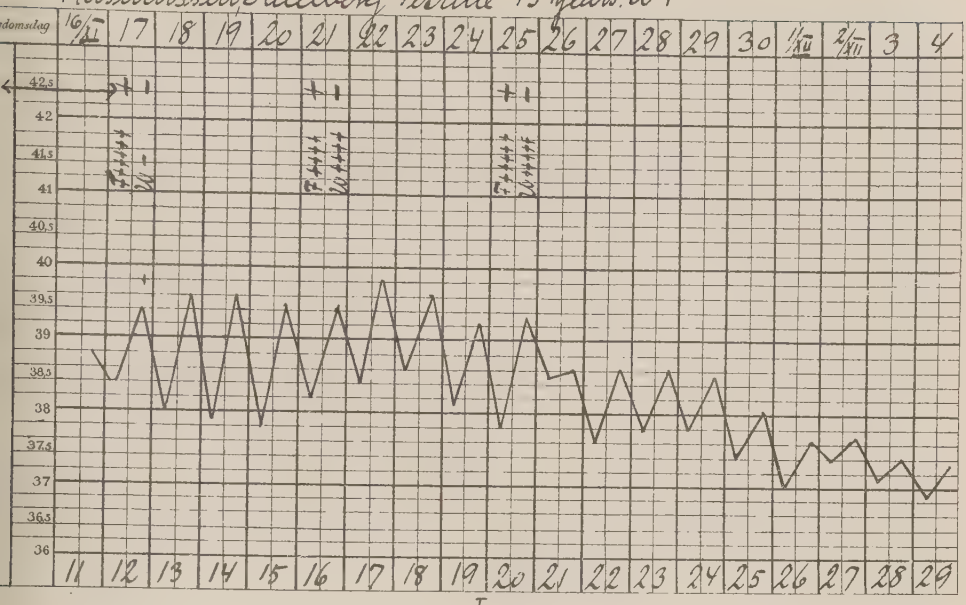
F: feces.

U: urine.

The nethermost row of figures shews the day of illnes.

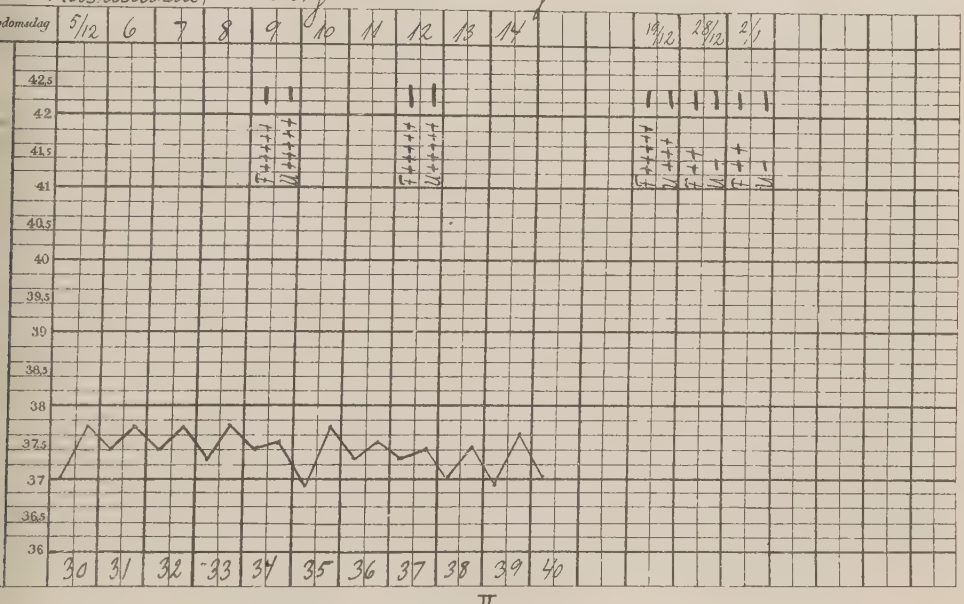
Temperatures are shewn in Celsius.

Rasmussen, Hillerød, Petrine 15 years. W+



I

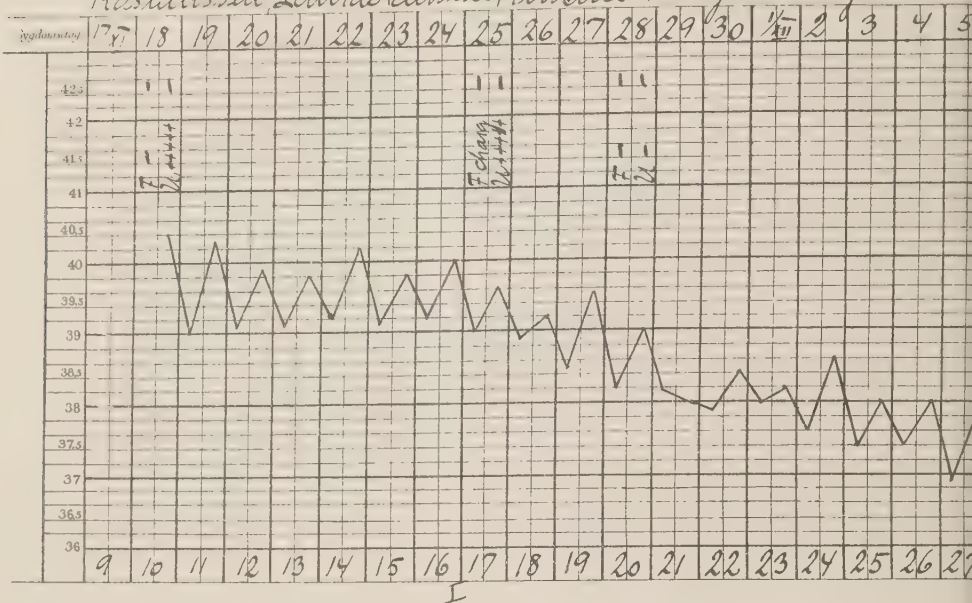
Rasmussen, Hillerød, Petrine. 15 years. W+



II

1

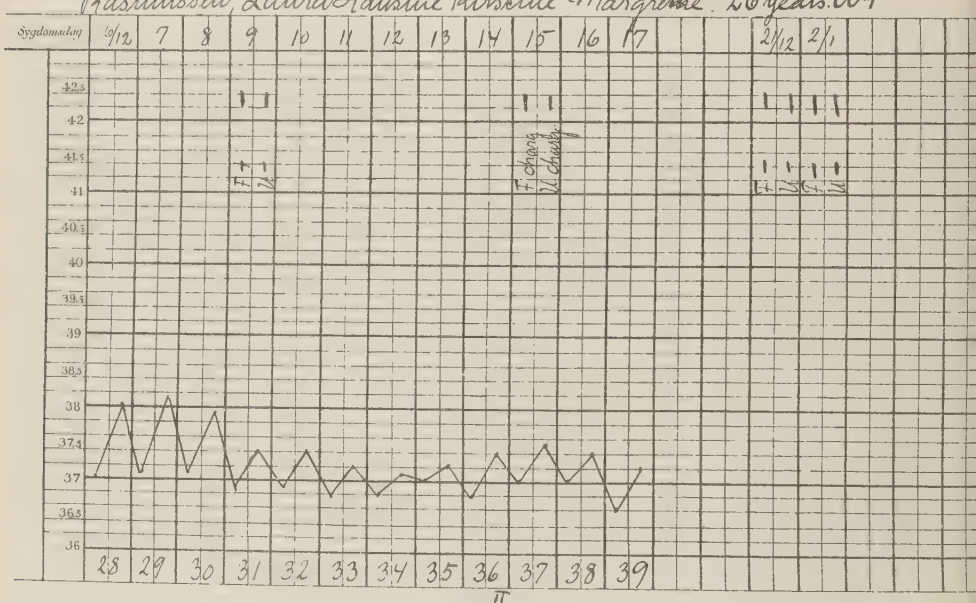
Rasmussen, Laura Hansine Kirstine Margrethe. 26 years. W+



I

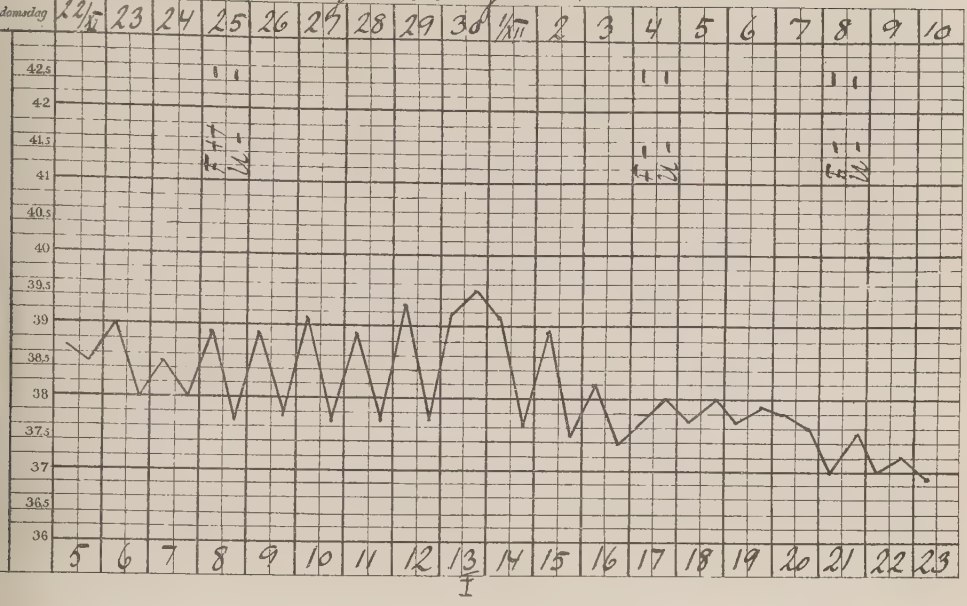
2

Rasmussen, Laura Hansine Kirstine Margrethe. 26 years. W+

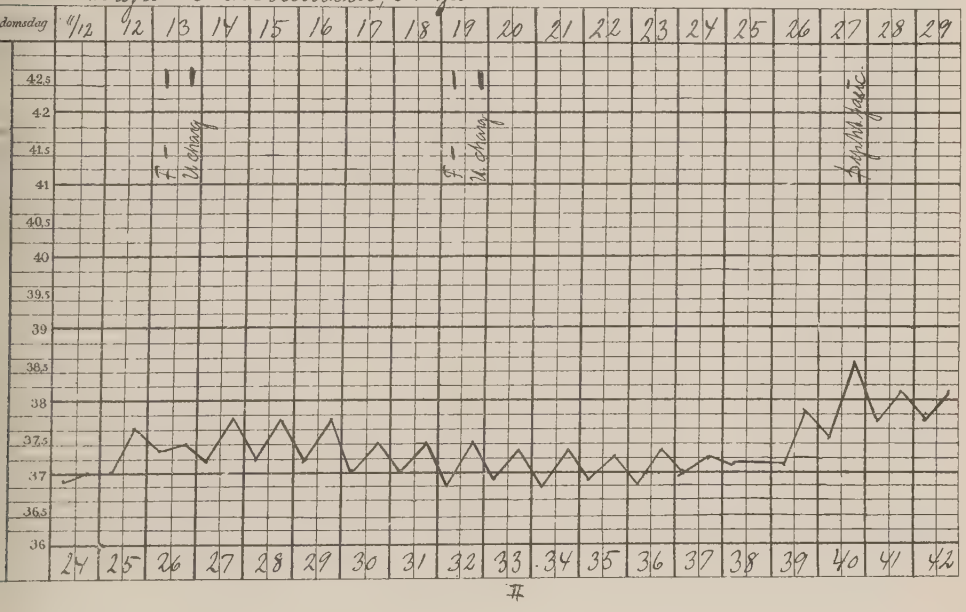


II

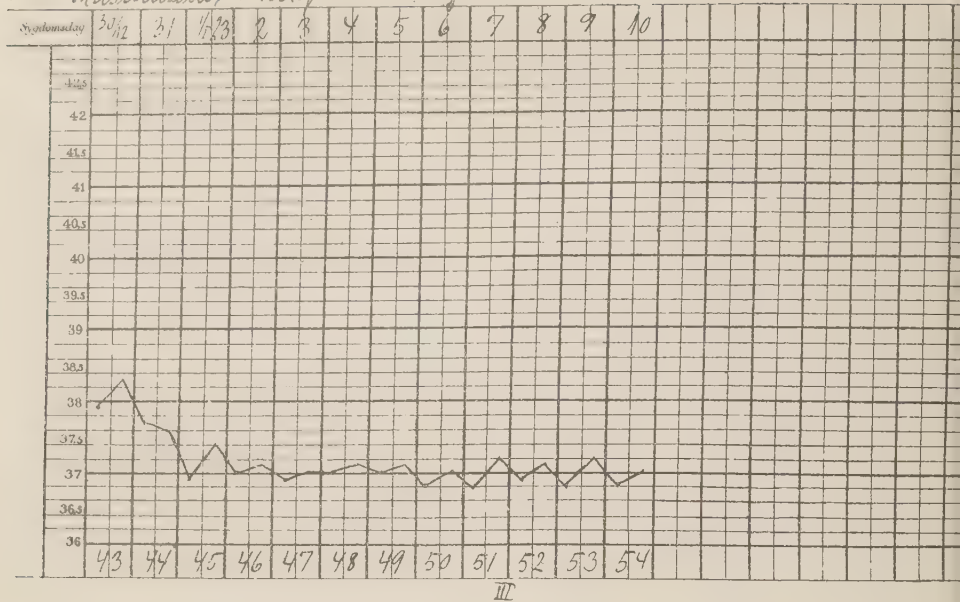
Rasmussen, Margrethe 65 years. W+



Margrethe Rasmussen, 65 years. W+ B.



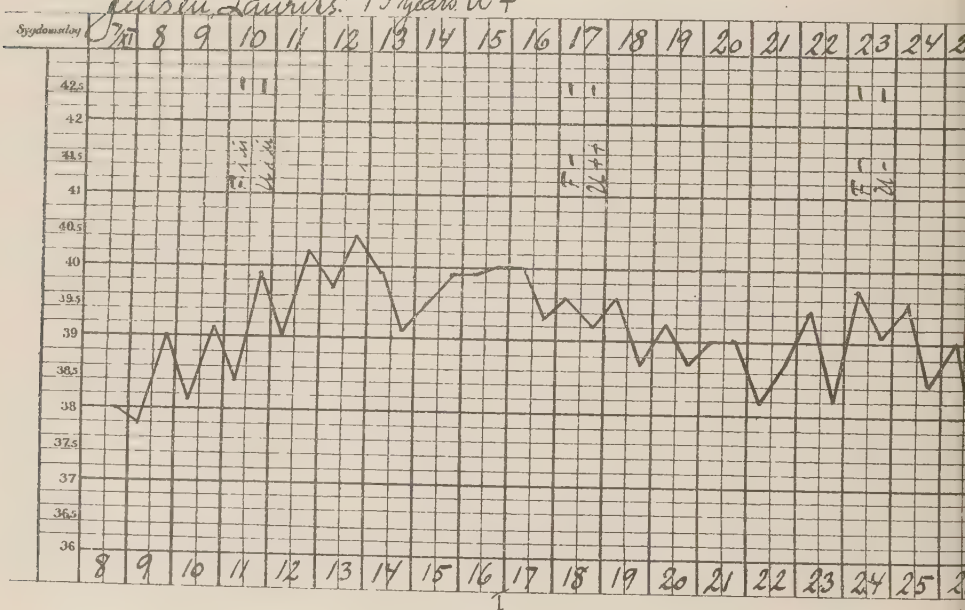
3 Rasmussen, Margrethe. 65 years W+



III

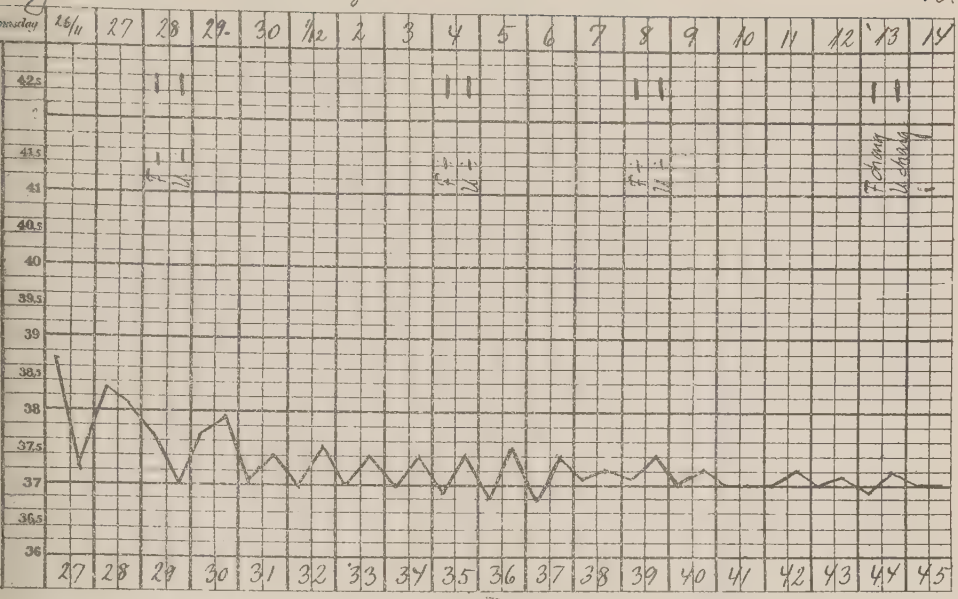
4

Jensen, Laurits: 43 years W+



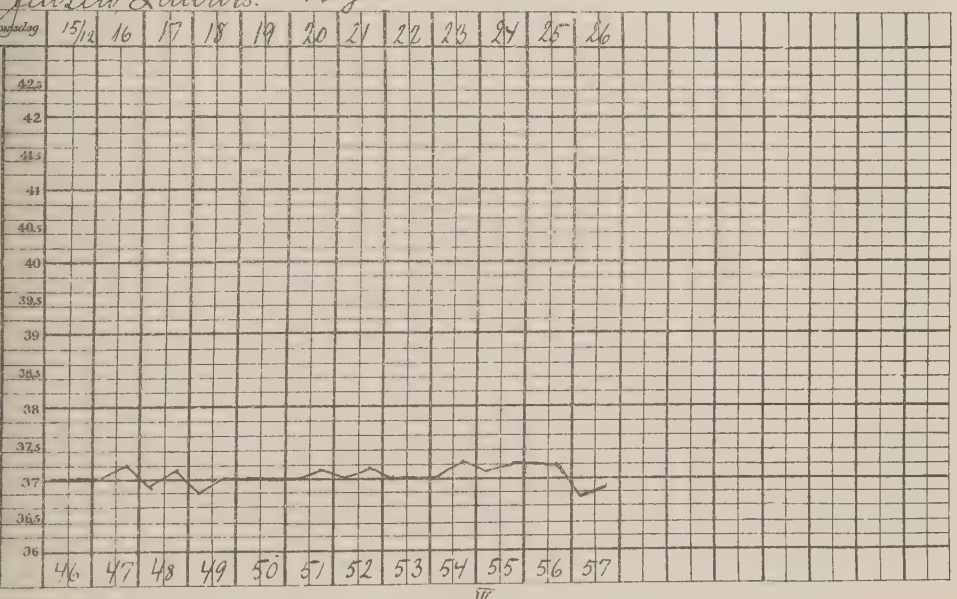
1

Jansen, Laurits 43 years. W+ B.



II

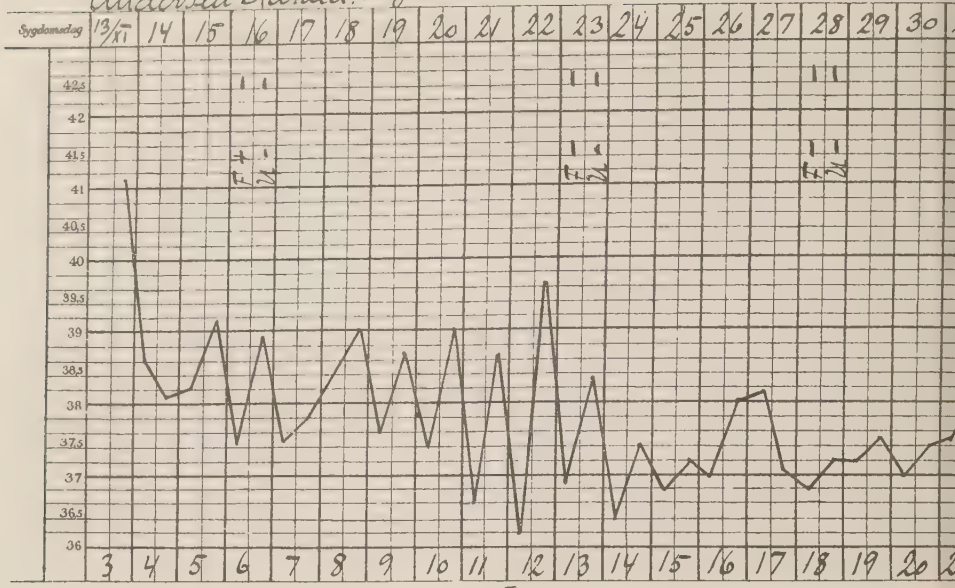
Jansen, Laurits. 43 years. W+ B



III

5

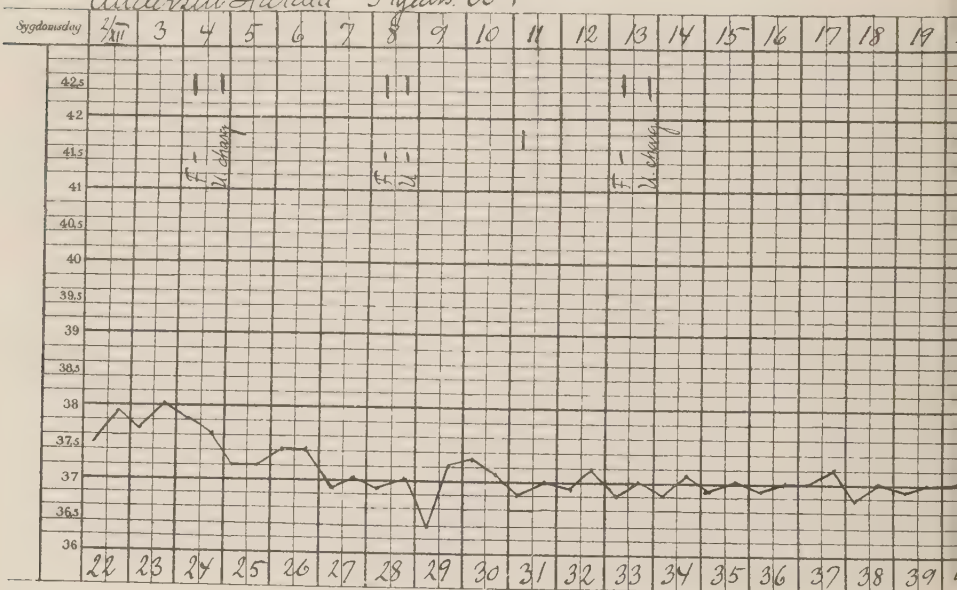
Andersen Harald. 3 years. W +



I

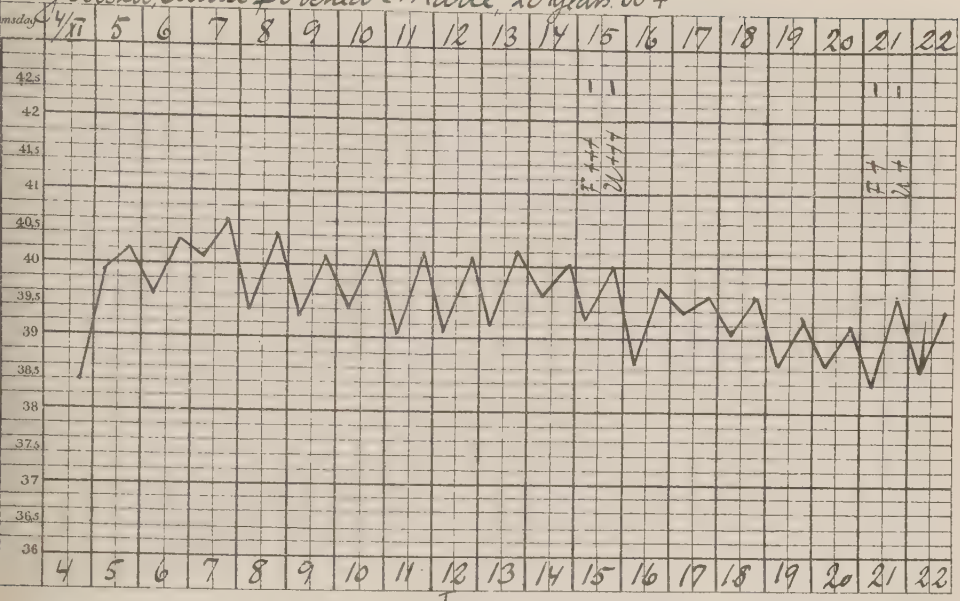
5

Andersen Harald 3 years. W +



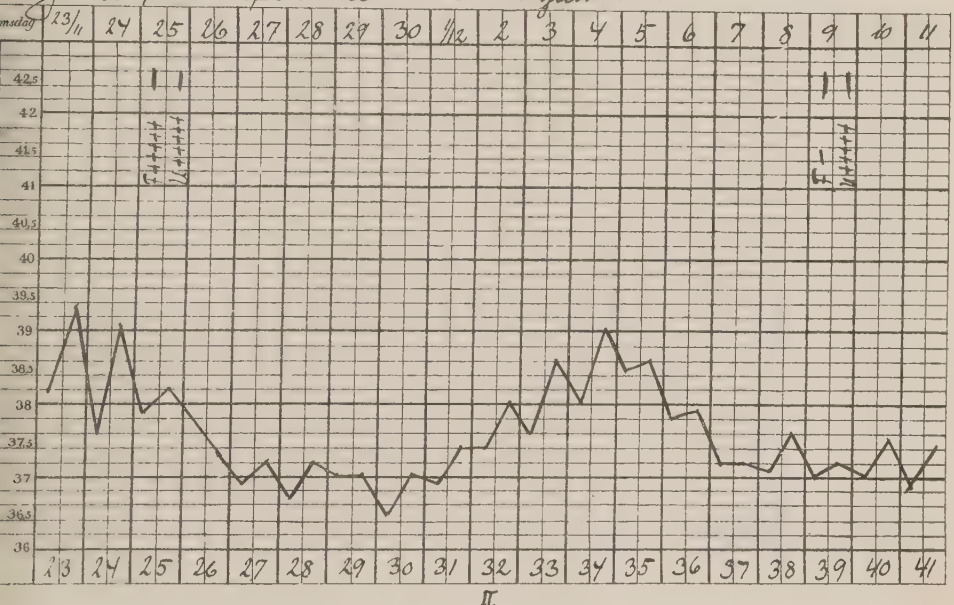
II

Jansen, Anna Dorthea Marie 20 years W +



I

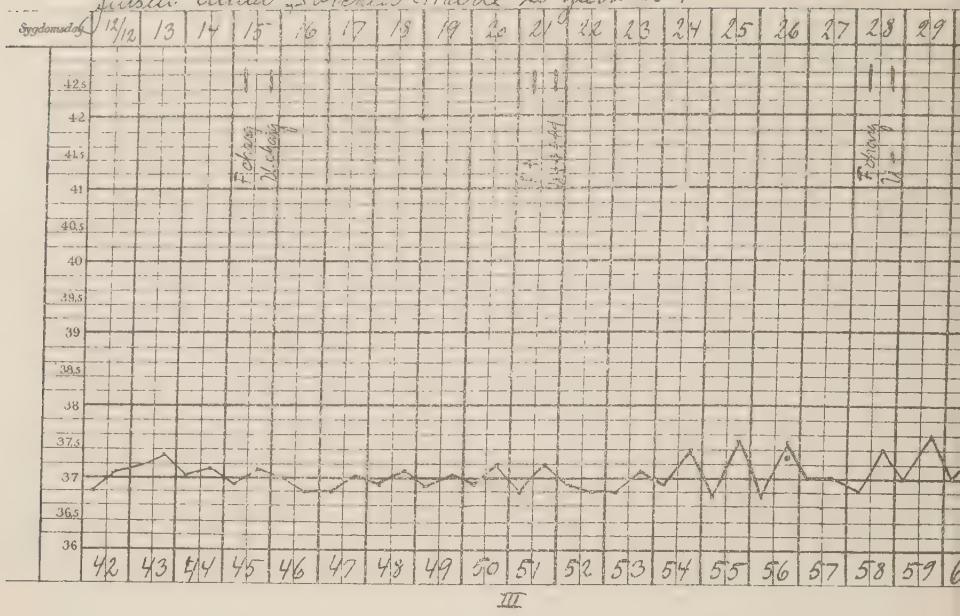
Jansen, Anna Dorthea Marie 20 years W +



II

6

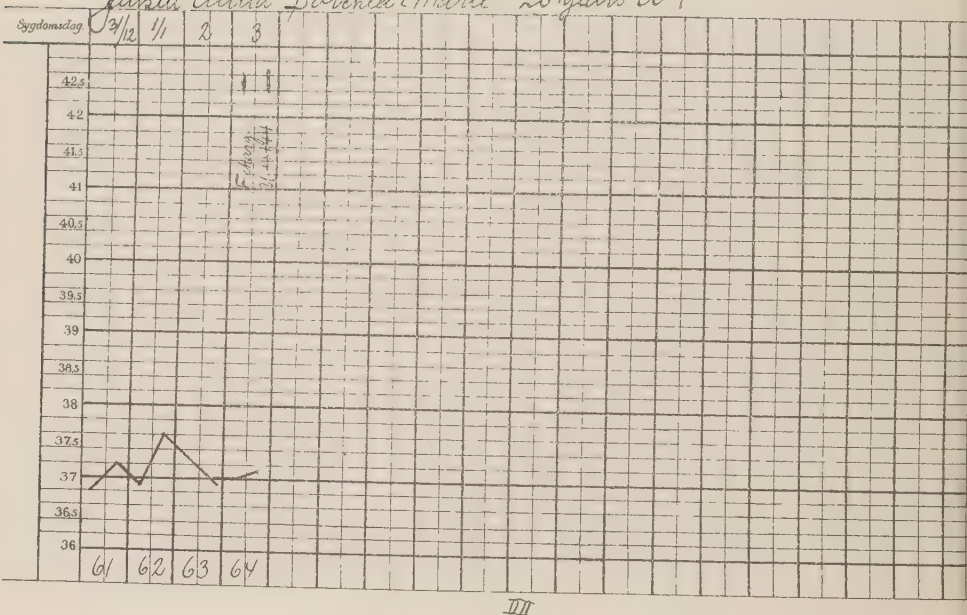
Jansen Anna Barbara Maria 20 years W +



III

6

Jansen Anna Barbara Maria 20 years W +



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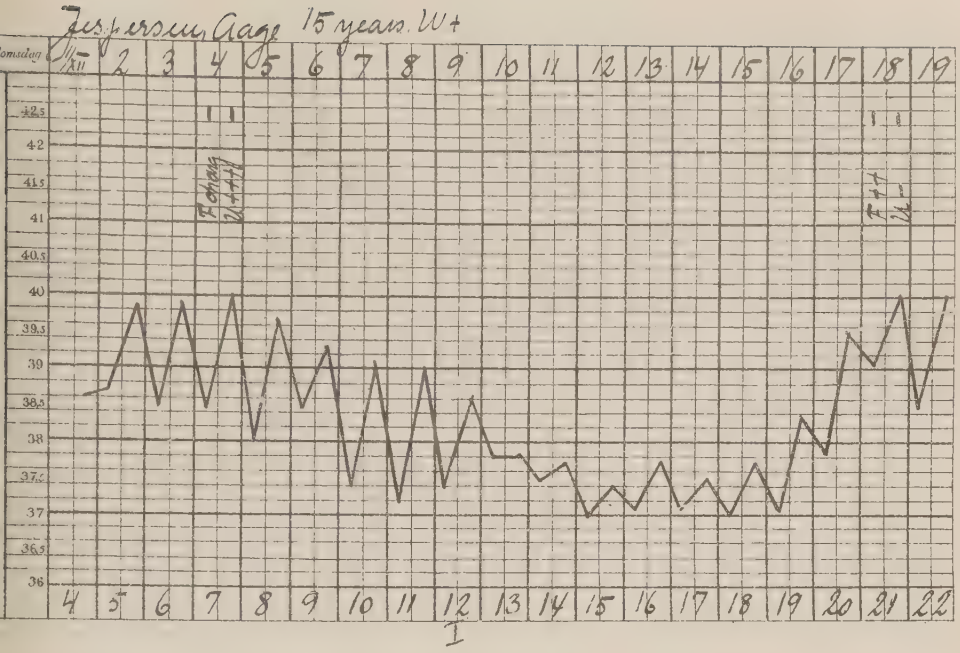
Jaspersen Aage 15 years W+
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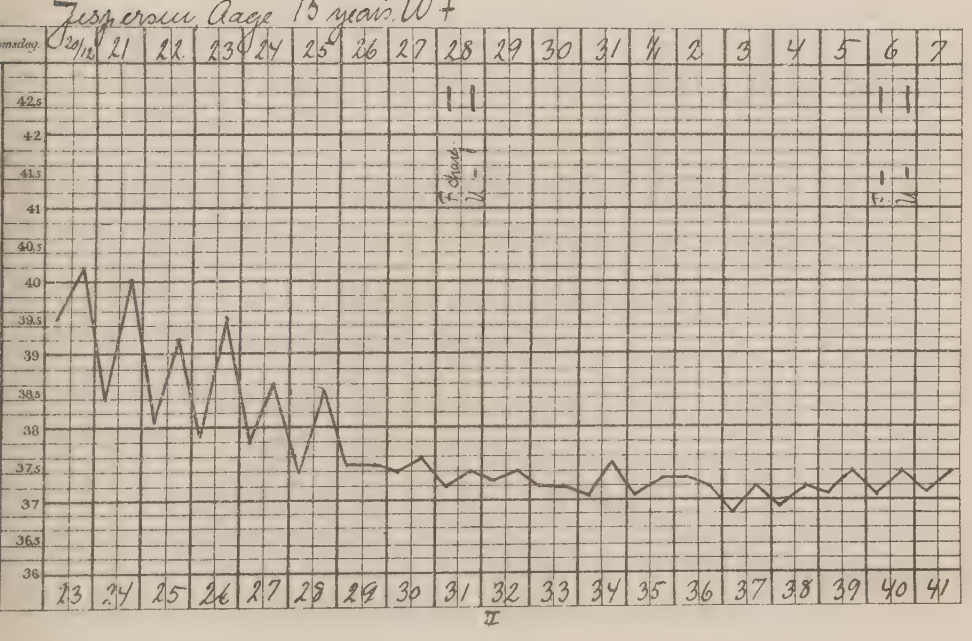
Jaspersen Aage 15 years W+
Tomsdag
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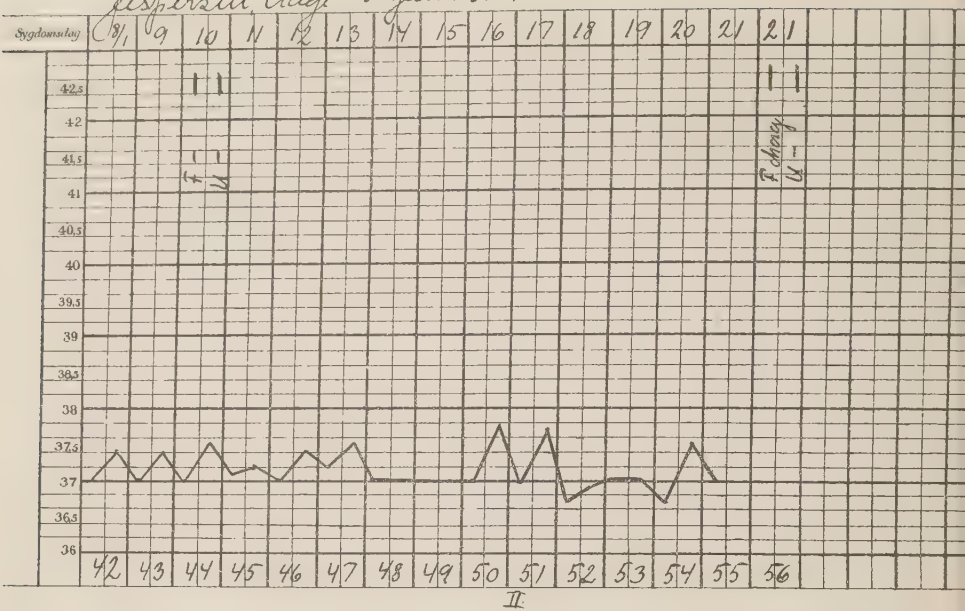
7

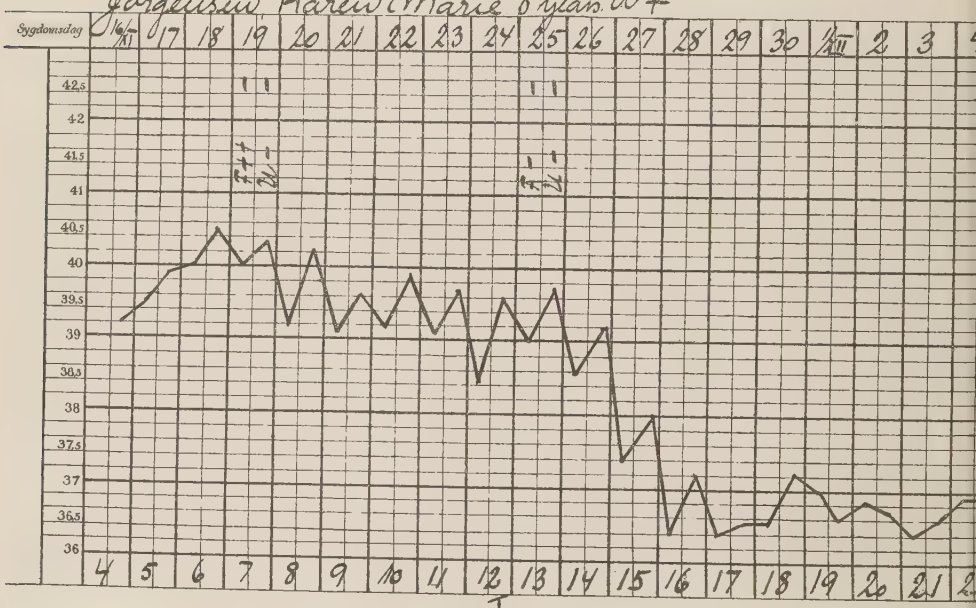
Jespersen, Aage 15 years. W+



II

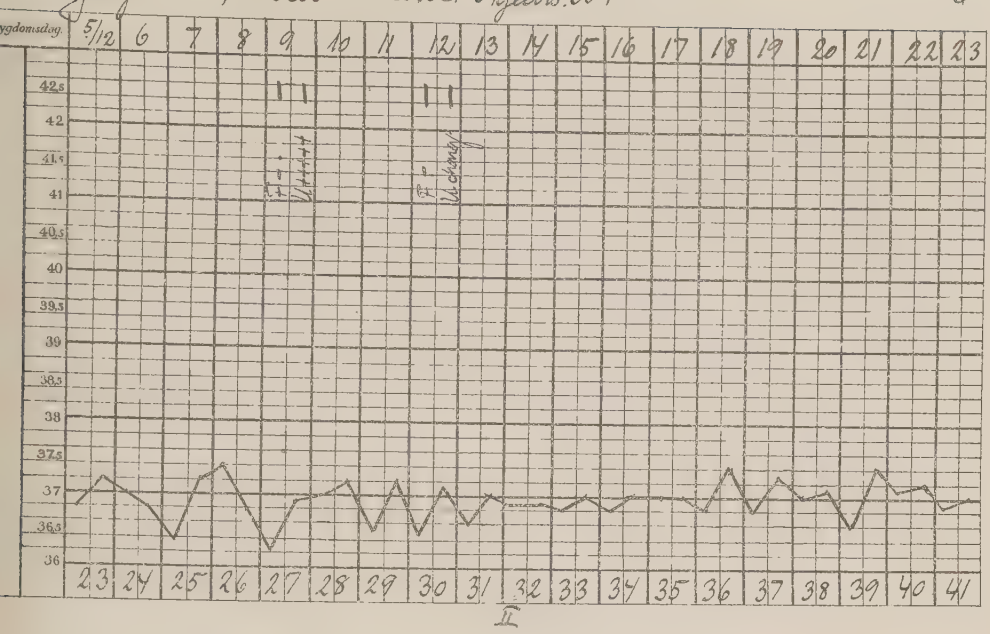
8

Jørgensen, Karen Marie 8 years. W+

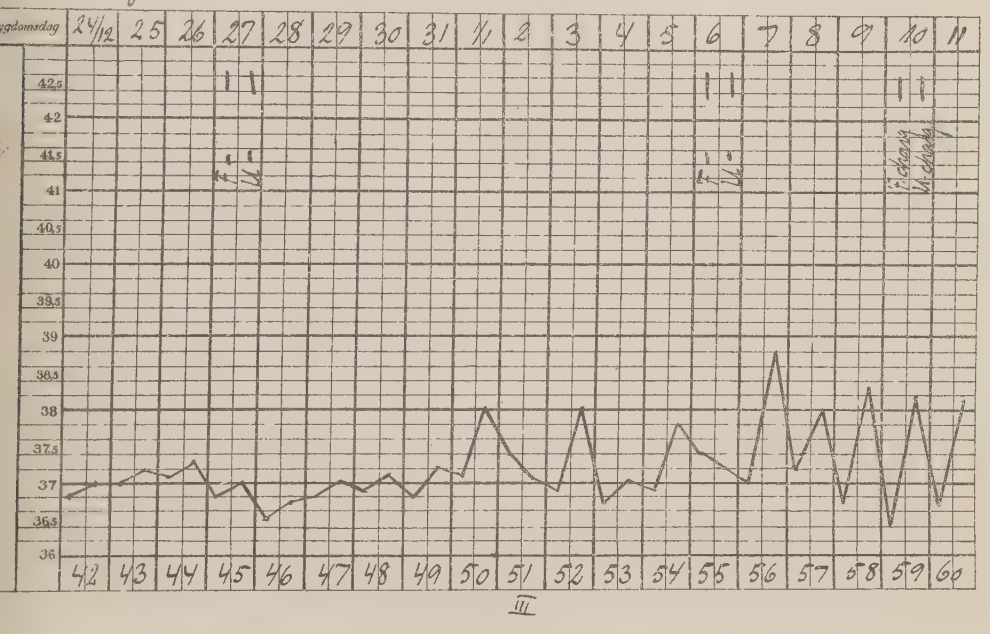


I

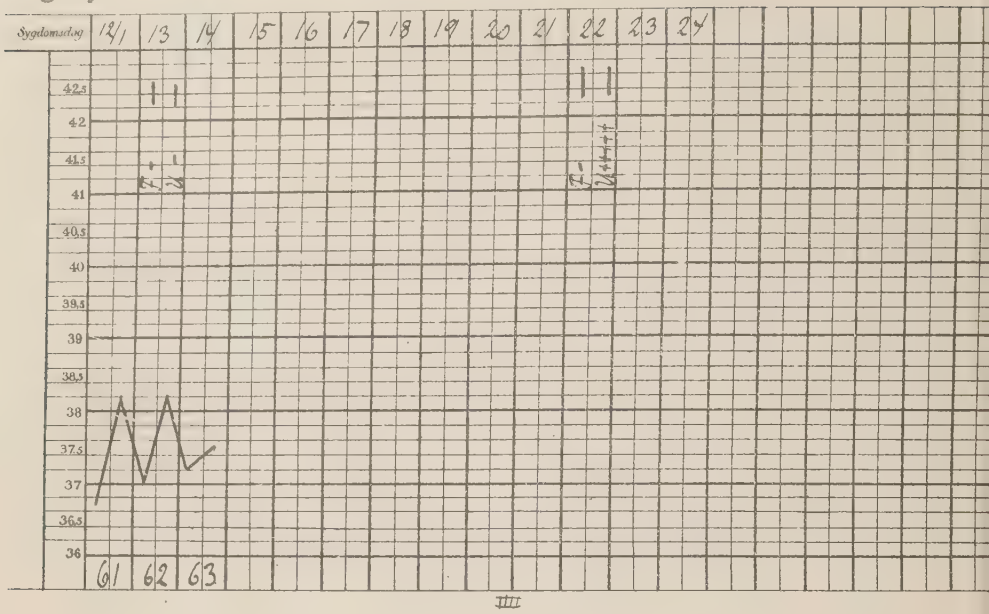
Jörgensen, Karen Marie. 8 years. W+ 0



Jörgensen, Karen Marie. 8 years. W+ 0.



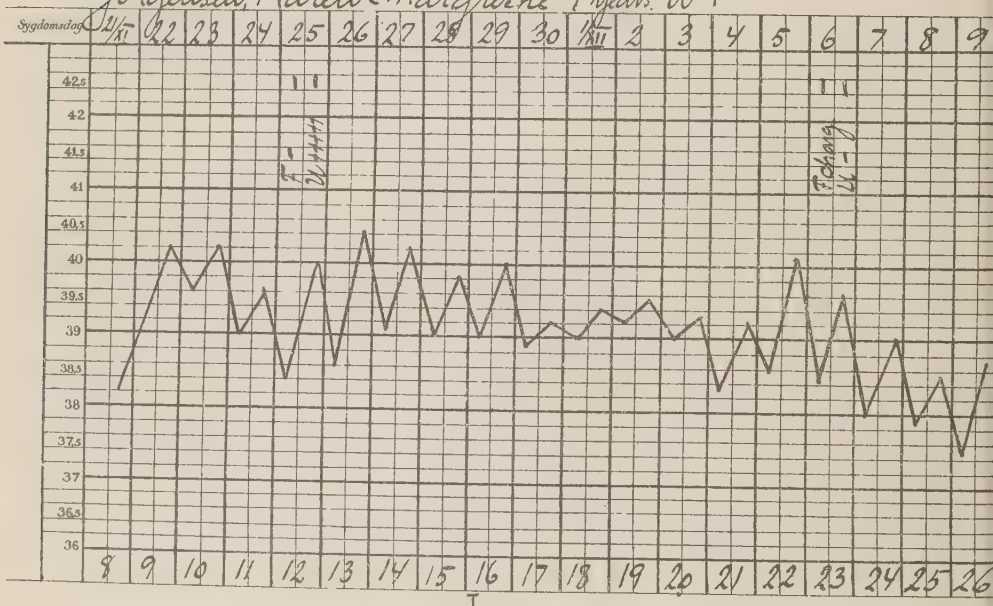
8 Jörgensen, Karen Marie. 8 years. W+



IIII

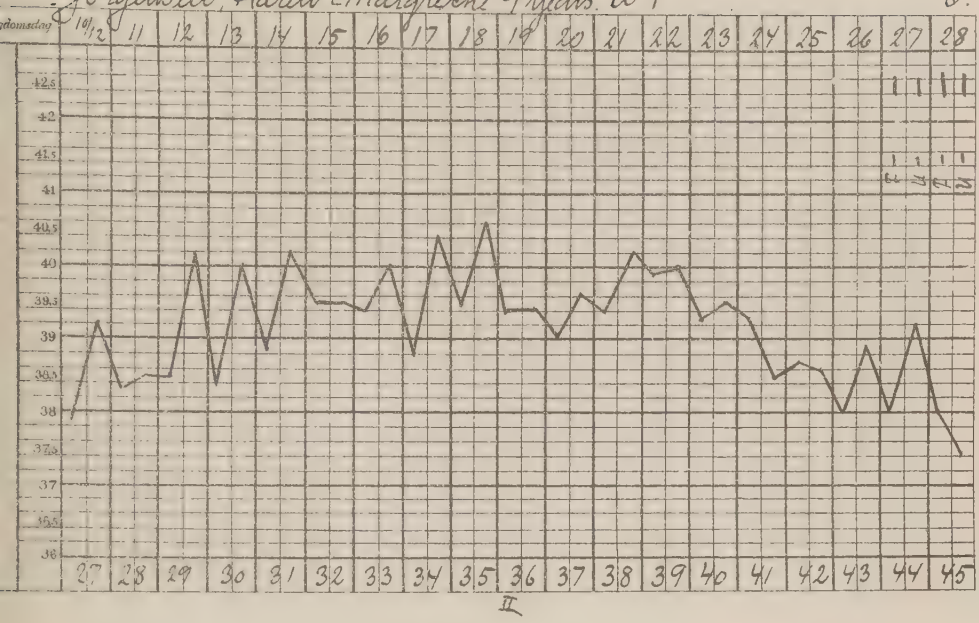
9

Jörgensen, Karen Margrethe. 9 years. W+

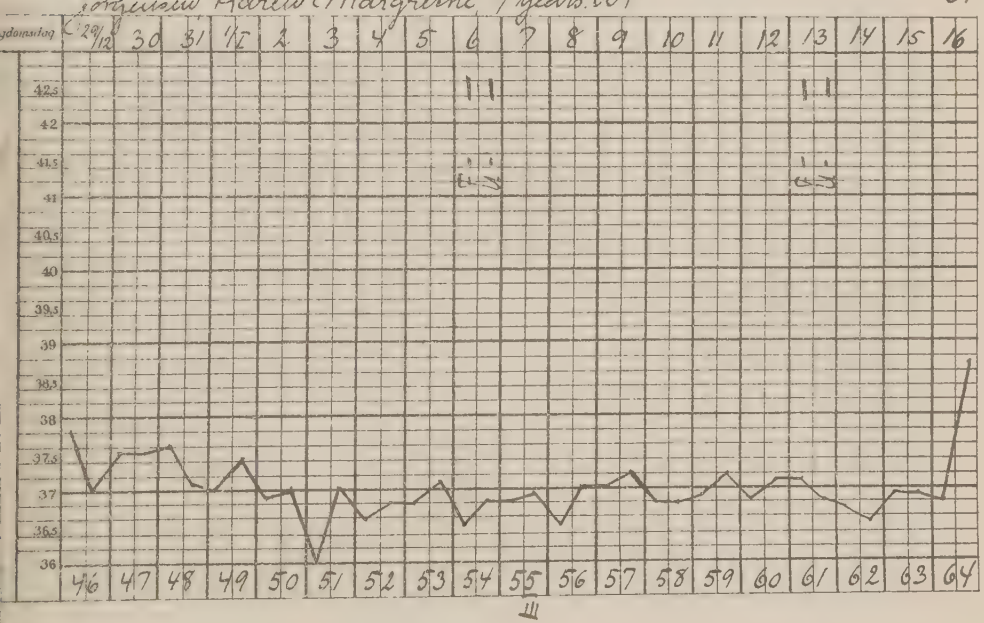


I

Jørgensen, Karen Margrethe 9 years. W+ O.

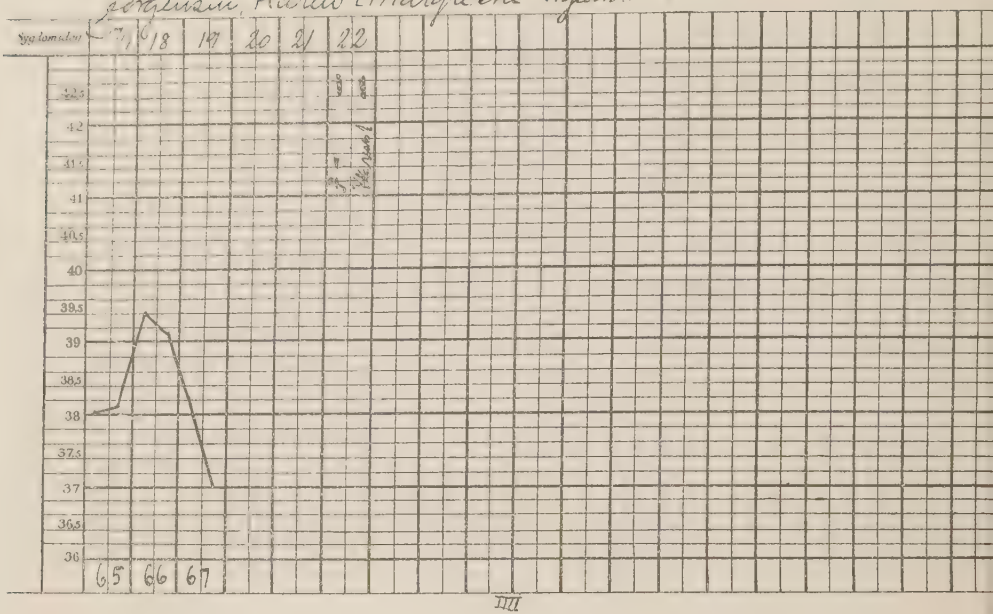


Jørgensen Karen Margrethe 9 years. W+ O.



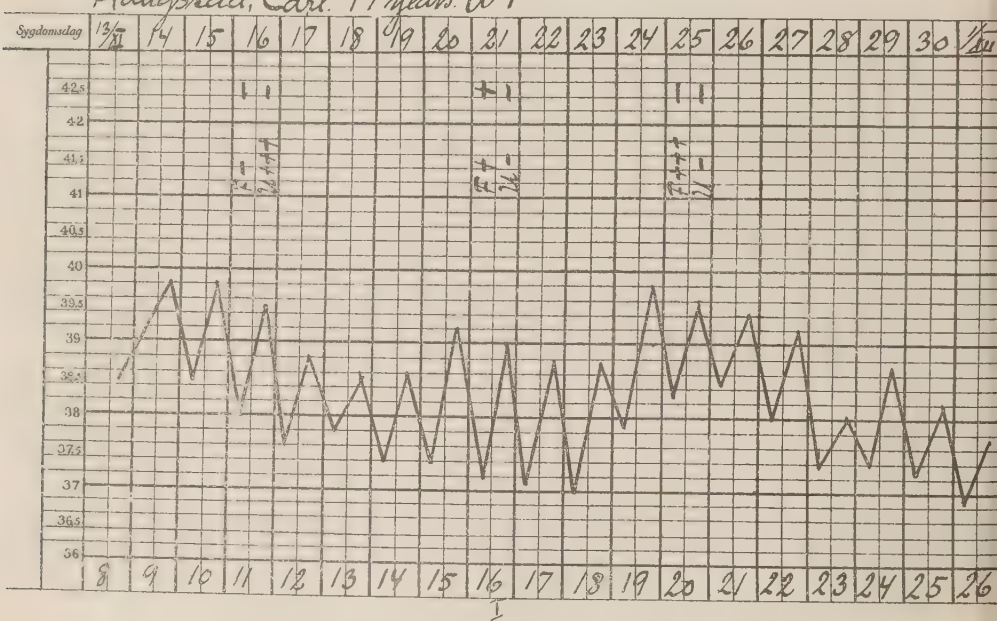
9

Jörgensen, Karin Margrethe 9 years. W+



10

Haugstad, Carl. 47 years. W+



Kongsbad. Carl. 47 years. W+

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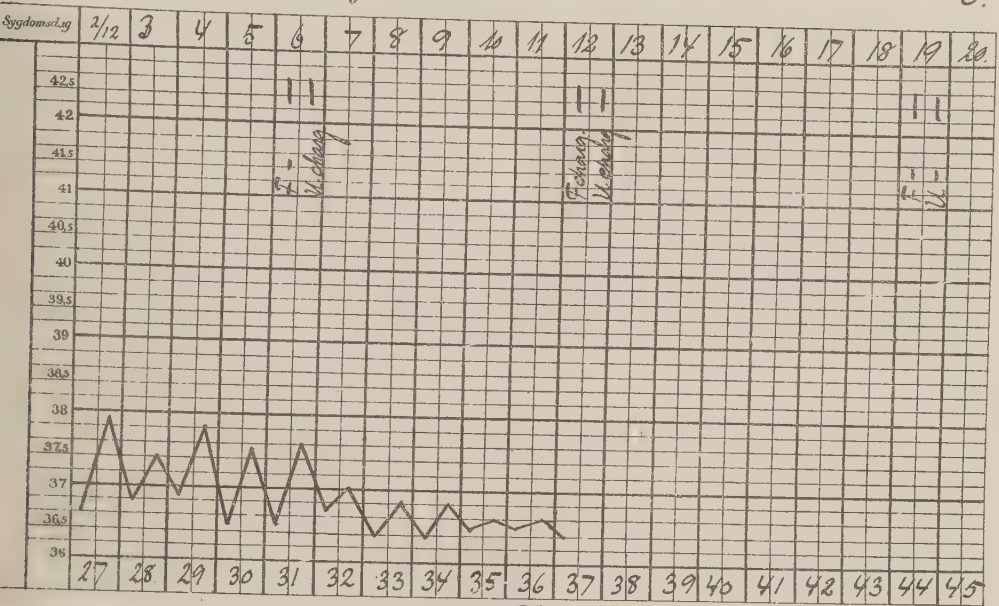

II

Kongsbad. Carl. 47 years. W+

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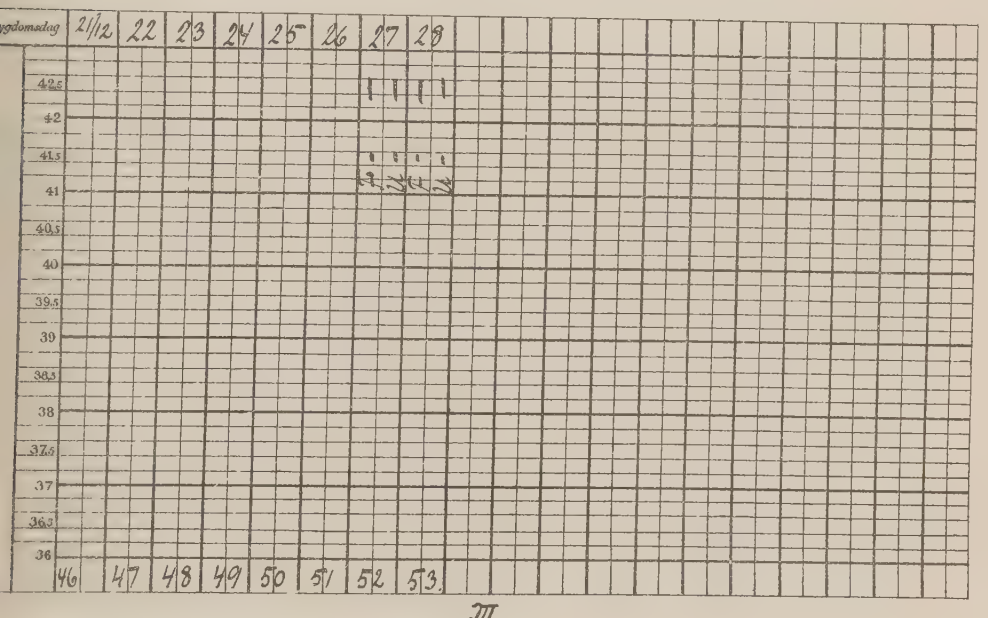
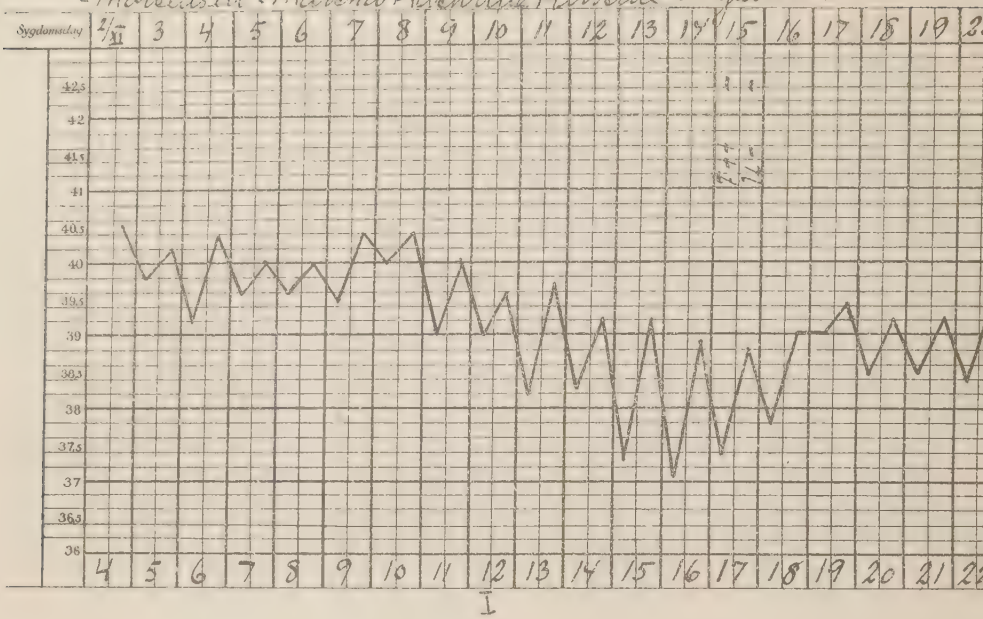


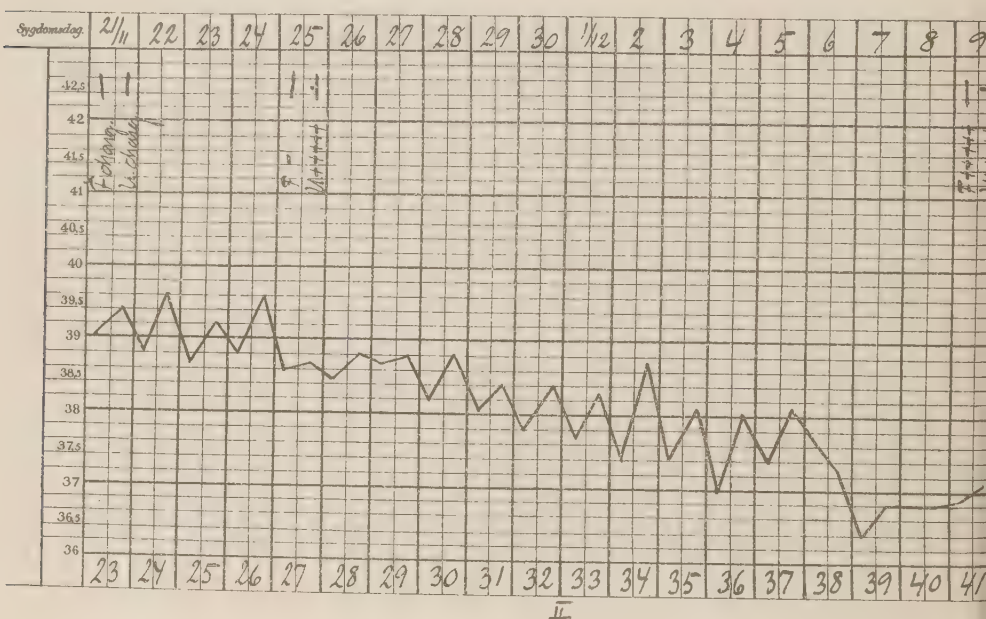
III

II

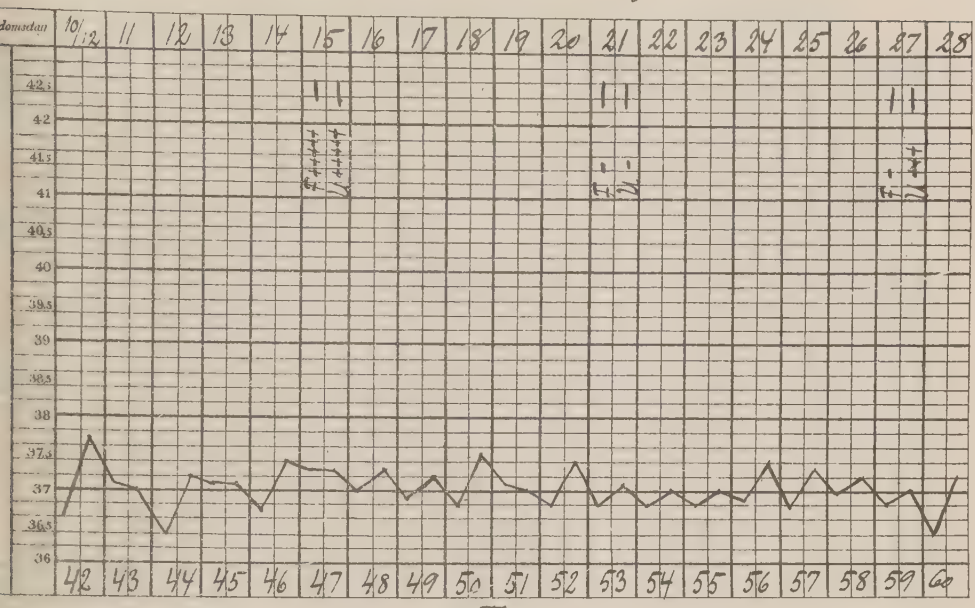
Marcussen Martha Kathrine Kirstine 35 years W+



II Marcussen, Martha Kathrine Kirstine. 35 years. W+

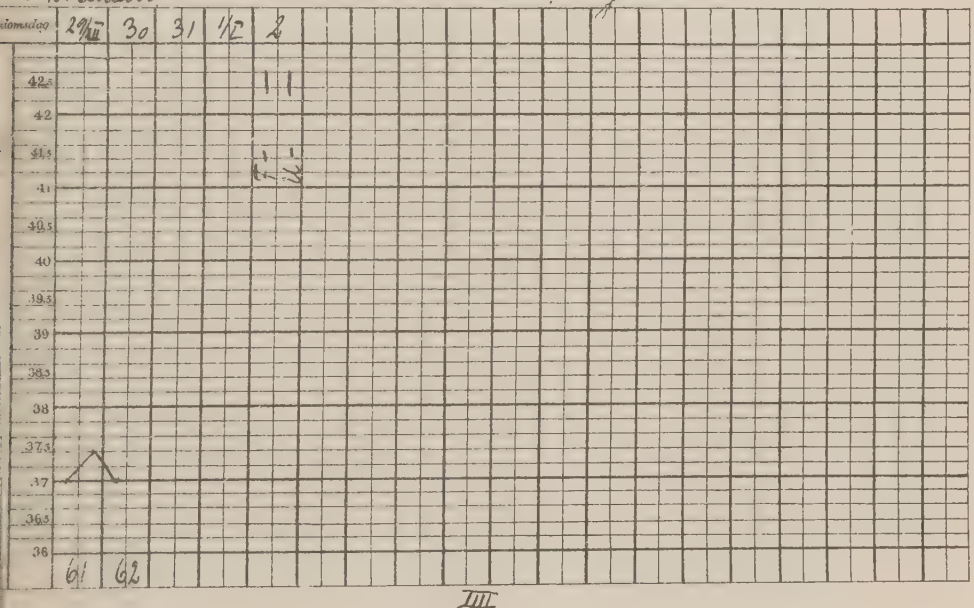


Mortensen, Martha Kathrine Kirstine 35 years W+ Q



III

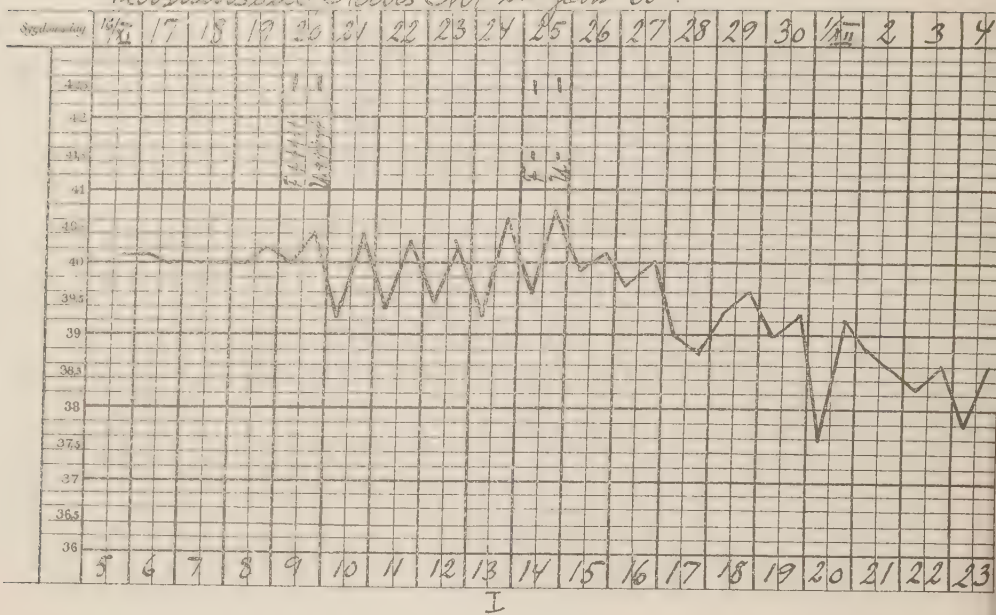
Mortensen Martha Kathrine Kirstine 35 years W+ Q.



IIII

12

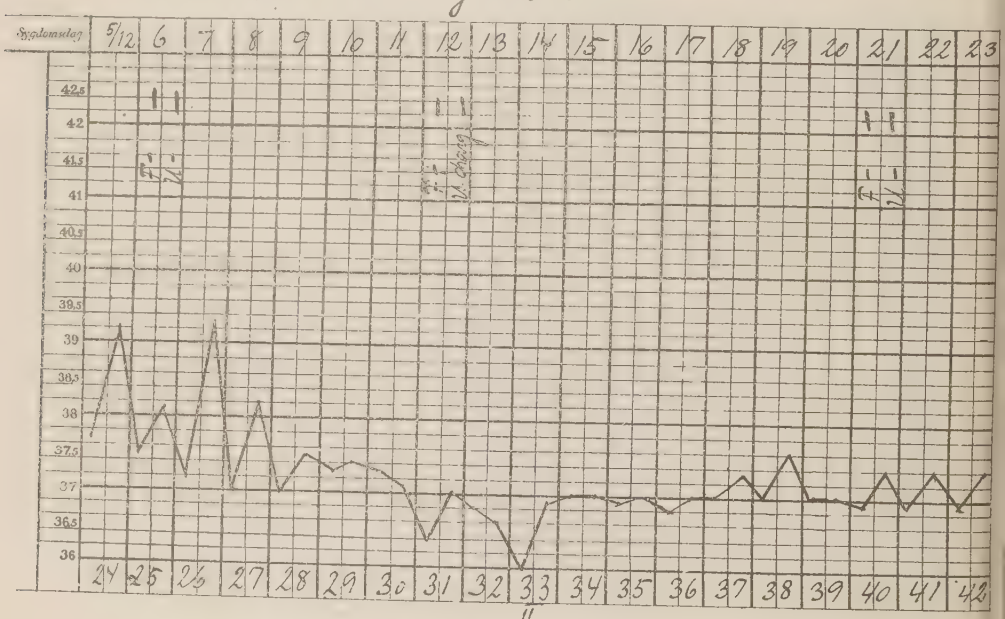
Rasmussen Hans Chr 22 years W+



I

12

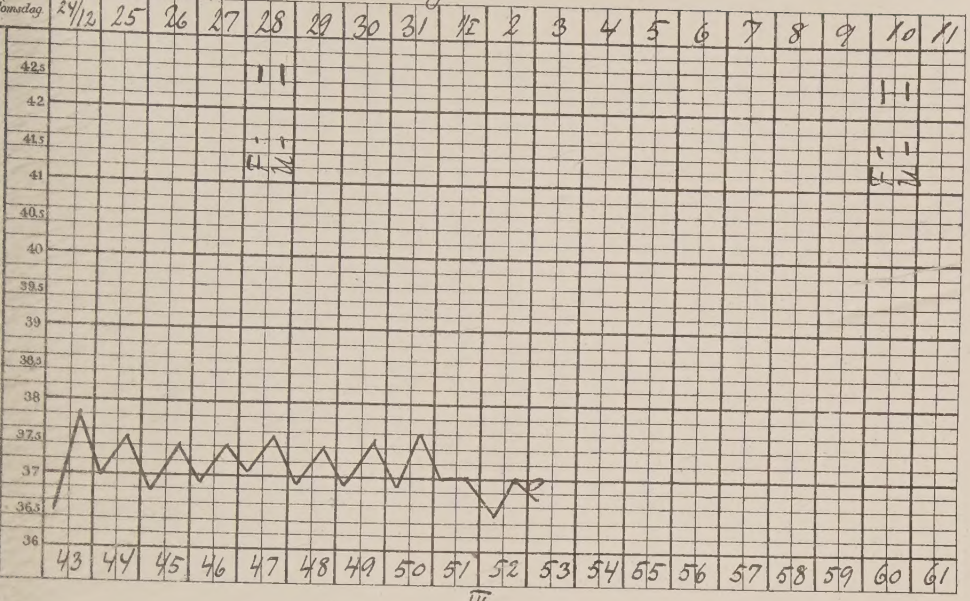
Rasmussen Hans Chr 22 years W+



II

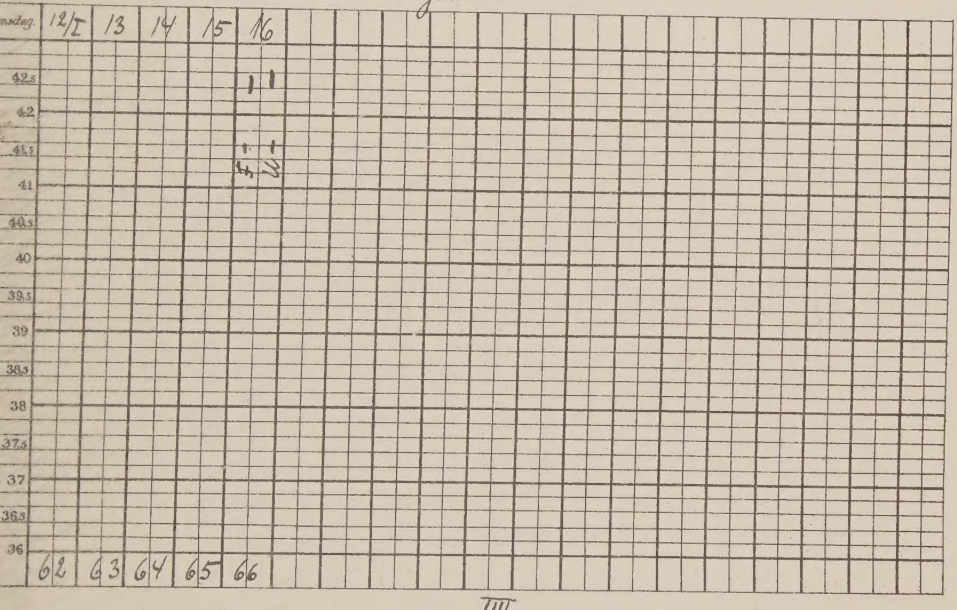
Rasmussen, Hans Chr. 22 years. W+

D.



III

Rasmussen, Hans Chr. 22 years. W+



IIII

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